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HETEROLOGOUS POLYPEPTIDE PRODUCTION IN THE ABSENCE OF NONSENSE-MEDIATED mRNA DECAY FUNCTION

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The invention relates to the discovery of a gene, *NMD2*, named after its role in the Nonsense-Mediated mRNA Decay pathway, and the protein, Nmd2p, encoded by the *NMD2* gene. The amino acid sequence of Nmd2p and the nucleotide sequence of the *NMD2* gene encoding it are disclosed. Nmd2p is shown herein to bind to another protein in the decay pathway, Upf1p. A C-terminal fragment of the protein is also shown to bind Upf1p and, when overexpressed in the host cell, the fragment inhibits the function of Upf1p, thereby inhibiting the nonsense-mediated mRNA decay pathway. The invention also relates to methods of inhibiting the nonsense-mediated mRNA decay pathway to stabilize mRNA transcripts containing a nonsense codon which normally would cause an increase in the transcript decay rate.

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HETEROLOGOUS POLYPEPTIDE PRODUCTION IN THE ABSENCE OF NONSENSE-MEDIATED mRNA DECAY FUNCTION

Statement as to Federally Sponsored Research

This invention was made at least in part with funds from the Federal government, and the government therefore has rights in the invention.

Background of the Invention

It is well known in the field of biology that changes in the amino acid sequence of a protein can result in changes in the biological function of the protein. To optimize a target biological function, the amino acid sequence can be altered and tested for improved function. In very simple terms, this is the process of evolution by which the proteins that exist naturally today have been selected over eons. It is an advantage of modern molecular biology that such alterations can be made in a matter of days rather than a matter of centuries. Specifically, optimizing the biological function of a protein of pharmaceutical or other commercial interest can be performed by substituting one amino acid for the naturally occurring amino acid at a given site and producing a sufficient quantity of the protein for screening of biological activity.

Production of a recombinant protein in a cellular system requires the efficient translation of the mRNA transcript encoding the protein. For this to occur, the transcript must exist in the cell long enough for translation into the desired recombinant protein. mRNA transcripts vary in the length of time (transcript half-life) that they exist in a cell prior to being degraded by cellular proteins specific for that purpose. In some

cases, degradation occurs rapidly such that very little protein is produced.

For example, the yeast cell, *Saccharomyces cerevisiae*, a commonly used cellular system for the production of recombinant proteins, has a biological pathway that specifically degrades mRNA transcripts containing a non-coding triplet sequence (nonsense or stop codons) in the transcript. In several genes studied thus far, the destabilizing nonsense codon occurs within the 5'-proximal portion of the transcript (reviewed in Peltz et al., Prog. Nucl. Acids Res. and Mol. Biol. (1994) 47:271-297). The translation process stops at the nonsense codons prior to reaching the end of the transcript's coding sequence resulting in the production of a truncated protein that may not possess normal biological activity. Thus, the cell has developed a biochemical system to degrade transcripts containing mutations that create stop codons early in the coding sequence.

However, in a cell of a suppressor strain that suppresses nonsense codons, a nonsense codon can be a useful means of coding for an alternate amino acid when a nonsense codon is engineered into the coding sequence to produce an altered protein which is then screened for enhanced biological activity. Suppressor strains (e.g., *SUF1-1*) do not allow maximal expression of a nonsense codon-containing transcript (Leeds et al., (1991) Genes & Development 5:2303-2314).

Nonsense-mediated mRNA decay is a phenomenon in which nonsense mutations, e.g., point or frame shift mutations that create a stop codon in the reading frame, in a gene can enhance the decay rate of the mRNA transcribed from that gene. For a review, see, e.g., Peltz et al., (1994) Prog. Nucleic Acid Res. Mol. Biol. 47:271-297. The process occurs in viruses, prokaryotes,

and eukaryotes (Leeds (1991), supra; Barker, G.F. and Beemon, K. (1991) Mol. Cell Biol. 11:2760-2768; Lim, S.-K. and Maquat, L.E. (1992) EMBO J 11:3271-3278).

In most genetic systems, 61 of the 64 possible codon triplets encode amino acids. The triplets UAA, UAG, and UGA are non-coding (nonsense codons) and promote translational termination (Osawa et al., (1992) Microbiol. Rev. 56:229-264). The polypeptide chain terminating effects of UAA, UAG, and UGA triplets have been amply documented and characterized (Craigen et al., (1990) Mol. Microbiol. 4:861-865).

Nonsense-mediated mRNA decay has been studied extensively in the yeast *Saccharomyces cerevisiae* where it has been shown that degradation of mRNA via this pathway is most likely to occur in the cytoplasm and is linked to translation. Evidence in support of these conclusions includes the following: 1) unstable, nonsense-containing mRNAs are stabilized in a strain harboring an amber suppressor tRNA (Losson and Lacroute, (1979) Proc. Natl. Acad. Sci. 76:5134-5137; Gozalbo and Hohmann, (1990) Curr. Genet. 17:77-79); 2) nonsense-containing mRNAs are ribosome-associated (Leeds et al., (1991) Genes & Devel 5:2303-2314; He et al., (1993) Proc. Natl. Acad. Sci. 90:7034-7039) and the number of ribosomes associated with such mRNAs is a function of the relative positions of the respective nonsense codons (He et al., (1993) Proc. Natl. Acad. Sci. 90:7034-7039); and 3) treatment of cells with cycloheximide, an inhibitor of translational elongation, stabilizes nonsense-containing mRNAs, yet removal of cycloheximide leads to the immediate restoration of rapid mRNA decay (Peltz et al., (1993) Genes & Devel 7:1737-1754).

Previous studies of nonsense-mediated mRNA decay in yeast also have shown that the products of the *UPF1* and *UPF3* genes (proteins Upf1p and Upf3p, respectively)

are essential components of this degradative pathway. Mutations in these genes stabilize mRNAs containing premature nonsense codons without affecting the decay rates of most wild-type transcripts (Leeds et al., (1991) Genes & Devel 5:2303-2314, Leeds et al., (1992) Mol. Cell. Biol. 12:2165-2177; Peltz et al., (1993) Genes & Devel 7:1737-1754; He et al., (1993) Proc. Natl. Acad. Sci. 90:7034:7039).

The *UPF1* gene has been cloned and sequenced (Leeds, P. et al., (1992) Mol. Cell Biol. 12:2165-2177), and shown to be: 1) non-essential for viability; 2) capable of encoding a 109 kD protein with a so-called zinc finger, nucleotide (GTP) binding site, and RNA helicase motifs (Leeds et al., (1992) Mol. Cell. Biol. 12:2165-2177; Altamura et al., (1992) J. Mol. Biol. 224:575-587; Koonin, (1992) Trends Biochem. Sci. 17:495-497); 3) identical to *NAM7*, a nuclear gene that was isolated as a high copy suppressor of mitochondrial RNA splicing mutations (Altamura et al., (1992) J. Mol. Biol. 224:575-587); and 4) partially homologous to the yeast *SEN1* gene (Leeds et al., (1992) Mol. Cell. Biol. 12:2165-2177). The latter encodes a noncatalytic subunit of the tRNA splicing endonuclease complex (Winey and Culbertson, (1988) Genetics 118:607-617; DeMarin et al., (1992) Mol. Cell Biol. 12:2154-2164), suggesting that the Upf1p protein (Upf1p) may also be part of a nuclease complex targeted specifically to nonsense-containing mRNAs.

Suppression of nonsense-mediated mRNA decay in *upf1* deletion strains does not appear to result simply from enhanced read-through of the termination signal (Leeds et al., (1991) Genes & Devel 5:2303-2314), nor does it appear to be specific for a single nonsense codon. The ability of *upf1*$^-$ mutants to suppress *tyr*7-1 (UAG), *leu*2-1 (UAA), *leu*2-2 (UGA), *met*8-1 (UAG), and *his*4-166 (UGA) (Leeds et al., (1992) Mol. Cell Biol.

12:2165-2177) indicates that they can act as omnipotent suppressors. *upf1*$^-$ mutants degrade nonsense-containing transcripts at a slower rate allowing synthesis of sufficient read-through protein to permit cells to grow under nutrient-deficient conditions that are nonpermissive for *UPF1*$^+$ cells.

Summary of the Invention

The invention relates to the discovery of a gene, *NMD2*, named after its role in the Nonsense-Mediated mRNA Decay pathway, and the protein, Nmd2p, encoded by the *NMD2* gene. Nmd2p is shown herein to bind to Upf1p. A C-terminal fragment of the protein is also shown to bind Upf1p and, when overexpressed in the host cell, the fragment inhibits the function of Upf1p, thereby inhibiting the nonsense-mediated mRNA decay pathway.

The invention further relates to the inhibition of the nonsense-mediated mRNA pathway to produce a heterologous recombinant protein or polypeptide in a host cell or to increase the production of an endogenous protein useful to a host cell or organism. A codon of the gene encoding the recombinant protein is mutated to encode a nonsense codon. Expression of this recombinant protein is enhanced by stabilizing the nonsense codon-containing mRNA transcript in a host cell in which the nonsense-mediated mRNA decay pathway is inhibited.

The insertion of a nonsense codon into the gene of interest is useful to produce an altered heterologous protein by amino acid substitution at the nonsense codon in a suppressor host strain. Insertion of a nonsense codon further allows the controlled expression of a protein that may be toxic to the cell by controlling the timing of nonsense mediated mRNA decay pathway inhibition. Insertion of a nonsense codon also allows the production of an N-terminal fragment of a

heterologous protein in increased yield when the nonsense codon-containing transcript is expressed in a host strain that is not a suppressor of nonsense codons.

It is an object of the invention to increase expression of nonsense codon-containing transcripts by inhibiting the nonsense-mediated mRNA decay pathway by overexpressing the C-terminal fragment of Nmd2p in the same cell that is also expressing the heterologous protein. Overexpression of the C-terminus of Nmd2p is not deleterious to the cell since its expression provides specific stabilization of transcripts having a stop codon early in the transcript and does not affect the stability of other transcripts.

The invention features a method of substantially inhibiting the nonsense-mediated mRNA decay pathway by providing a cell (such as a yeast cell) and mutating the *NMD2* gene such that essentially no functional Nmd2p is produced. For example, an insertional mutation which prevents synthesis of the Nmd2p results in an inhibited nonsense-mediated mRNA decay pathway without affecting the viability of the cell as described herein.

The invention features a method of substantially inhibiting the nonsense-mediated mRNA decay pathway by providing a cell (such as a yeast cell) and mutating the *UPF1* gene such that essentially no functional Upf1p is produced. For example, an insertional mutation which prevents synthesis of the Upf1p results in an inhibited nonsense-mediated mRNA decay pathway without affecting the viability of the cell as described herein.

The invention features a method of inhibiting the nonsense-mediated mRNA decay pathway by providing a cell and transforming the cell with a vector encoding *NMD2* operably linked to regulatory sequences for constitutive or inducible expression of the antisense transcript. Such an antisense transcript hybridizes to essentially

all of the *NMD2* sense transcript preventing translation and the production of functional Nmd2p, thereby inhibiting the nonsense mediated mRNA decay pathway. By "hybridizing to essentially all of the sense *NMD2* transcript" is meant that a sufficient amount of the sense transcript is bound by antisense transcript to inhibit translation such that substantially no functional Nmd2p is produced.

The invention features a method of inhibiting the nonsense-mediated mRNA decay pathway by providing a cell and transforming the cell with a vector encoding *UPF1* operably linked to regulatory sequences for constitutive or inducible expression of the antisense transcript. Such antisense transcript hybridizes with essentially all of the *UPF1* sense transcript preventing translation production of functional Upf1p, thereby inhibiting the nonsense mediated mRNA decay pathway. By "hybridizing to essentially all of the sense *UPF1* transcript" is meant that a sufficient amount of the sense transcript is bound by antisense transcript to inhibit translation such that substantially no functional Upf1p is produced.

The invention also features a substantially pure DNA of the *NMD2* gene, and degenerate variants thereof, involved in the nonsense-mediated mRNA pathway of a cell. The DNA of the invention is at least approximately 90% identical to SEQ ID NO:1 at the nucleotide level, and is preferably from the yeast *Saccharomyces cerevisiae*. The DNA encodes an amino acid sequence of Nmd2p (SEQ ID NO:2). The sequence of the invention is at least approximately 90% identical to the amino acid sequence of SEQ ID NO:2 at the amino acid level.

The invention also features the substantially pure DNA sequence of the 3′ terminus (SEQ ID NO:3) of *NMD2*. The 3′ terminus encodes the carboxy terminal fragment (SEQ ID NO:4) of Nmd2p, which fragment, when

overexpressed in a yeast cell, binds to Upf1p and inhibits the nonsense-mediated mRNA decay pathway.

In addition, the invention features a vector containing a DNA sequence (SEQ ID NO:1) encoding a polypeptide (SEQ ID NO:2). Preferably the coding sequence is under the transcriptional control of regulatory sequences that are activated and deactivated by an externally applied condition such as temperature, or an externally supplied chemical agent. Such control expression systems are well known to those of ordinary skill in the art. Thus, the expression of the DNA is turned on and off as necessary for the controlled (i.e. conditional) inhibition of the nonsense-mediated mRNA pathway.

The invention further features a vector containing a DNA sequence (SEQ ID NO:3) encoding a polypeptide (SEQ ID NO:4) which polypeptide, when overexpressed in a cell, inhibits the nonsense mediated mRNA pathway. Preferably the coding sequence is under the transcriptional control of regulatory sequences that are activated and deactivated by an externally applied condition such as temperature or an externally supplied chemical agent, controls expression systems well known to those of ordinary skill in the art. Thus, the expression of the DNA is turned on and off as necessary for the controlled (i.e. conditional) inhibition of the nonsense-mediated mRNA pathway.

The invention also features a host cell containing the DNA of SEQ ID NO:1 or SEQ ID NO:3 or fragments thereof. The invention also features cells harboring vectors containing the DNA of SEQ ID NO:1 or SEQ ID NO:3 or fragments thereof.

The invention features substantially pure nonsense-mediated mRNA decay protein, Nmd2p (SEQ ID

NO:2), and fragments thereof from a yeast cell, preferably from the genus *Saccharomyces*.

The invention also features a substantially pure nonsense-mediated mRNA decay protein Nmd2p C-terminal fragment (SEQ ID NO:4) and fragments thereof which bind to the nonsense-mediated mRNA decay pathway protein, Upf1p, and which when overexpressed in a cell, substantially inhibit nonsense-mediated mRNA decay in the cell.

The invention also features a cell containing a vector expressing a polypeptide containing the Nmd2p carboxy terminal fragment (SEQ ID NO:4), which fragment binds to the nonsense-mediated mRNA decay pathway protein, Upf1p and, when overexpressed in the cell, substantially inhibits nonsense-mediated mRNA decay in the cell.

In addition, the invention features methods of producing a heterologous polypeptide from an mRNA transcript in which the transcript contains at least one nonsense codon within a transcript destabilizing 5′ portion. The method involves providing a cell in which the nonsense mediated mRNA decay pathway is substantially inhibited by 1) overexpression of a polypeptide containing the Nmd2p carboxy terminal fragment (SEQ ID NO:4); or 2) mutation of *NMD2* or *UPF1* (e.g., insertional mutagenesis) resulting in inhibition of the nonsense-mediated mRNA decay pathway of the cell; or 3) expression of *NMD2* or *UPF1* antisense mRNA which hybridizes to the sense transcript of *NMD2* or *UPF1*, respectively, inhibiting translation and, thereby inhibiting nonsense mediated mRNA decay. Expression in this cell of a nonsense codon-containing gene encoding the heterologous polypeptide provides a transcript whose stability is enhanced at least 2 fold compared to a wild-type cell.

Translation of the transcript produces the heterologous polypeptide.

In another embodiment, the invention features antibodies that are raised against and bind specifically to Nmd2p, a protein having the amino acid sequence of SEQ ID NO:2, or a polypeptide having the amino acid sequence of SEQ ID NO:4. The antibodies can be polyclonal or monoclonal.

The invention further features a method of screening a candidate host cell for the presence or absence of 1) Nmd2p, 2) a C-terminal fragment of Nmd2p, 3) a polypeptide of SEQ ID NO:2, or 4) a polypeptide of SEQ ID NO:4, including fragments or analogs thereof. The method also can be used to determine relative amounts of each of the proteins in a cell. The screening method is useful for isolating a host strain in which heterologous protein production is to be optimized. The method first involves lysis of a clonal population of cells suspected of containing Nmd2p or Nmd2p fragment. Antibody to Nmd2p or Nmd2p fragment is contacted with proteins of the lysate. Presence, relative abundance, or absence of Nmd2p or Nmd2p fragment in the lysate is determined by the binding of the antibody. Possible detection methods include affinity chromatography, Western blotting, or other techniques well known to those of ordinary skill in the art.

It is an object of the invention that a heterologous polypeptide produced by the method of the invention can be a desired fragment of a protein or polypeptide. A nonsense codon is incorporated into the DNA sequence encoding the protein or polypeptide at a position within a transcript destabilizing 5′ portion of the sequence at a desired transcriptional stop site. Expression of the DNA in a cell having an inhibited nonsense-mediated mRNA decay pathway results in a

substantially increased half-life for the nonsense codon-containing transcript. An advantage of this method is the stabilization of the transcript allowing an increased amount of the protein fragment to be produced relative to the amount produced in a wild-type host strain.

As an object of the invention, a heterologous protein that is normally toxic to a cell is produced by controllably inhibiting the nonsense-mediated mRNA decay pathway and thereby, controlling the stability of a nonsense codon-containing transcript for the toxic protein. Inhibition of the nonsense-mediated mRNA decay pathway is accomplished, for example, by the inducible expression of the C-terminus of the Nmd2p only when protein production is desired (e.g., at optimal cell density of the culture). Inhibition of the nonsense-mediated mRNA decay pathway substantially increases the half-life of the transcript containing a nonsense codon in a transcript destabilizing 5′ portion of the transcript thereby increasing translation and production of the protein when desired. Preferably, in this feature of the invention, the cell expressing the heterologous protein is a nonsense suppressor cell in which the suppressor mechanism is controllably expressed and substitutes the naturally occurring amino acid at the site of a nonsense codon.

As an object of the invention, an altered heterologous polypeptide is produced in a nonsense suppressor cell by substituting an amino acid at the position of a nonsense codon, which amino acid is not the amino acid naturally occurring at that position. An amino acid is substituted which alters a target biological activity of the protein in the cell. The nonsense-mediated mRNA pathway is inhibited to increase production of the altered heterologous polypeptide from a

transcript containing a nonsense codon in a transcript destabilizing 5′ portion of the transcript.

Alteration in biological activity includes increased binding affinity to a target molecule such as a receptor, antibody, or decreased toxicity of the protein to the host strain in which the protein is produced. By "substantial reduction in toxicity" is meant that expression of the altered heterologous polypeptide allows the cell growth rate to be at least 2 fold greater than the growth rate in the presence of the natural toxic heterologous polypeptide, or allows sufficient cell growth for production of the altered heterologous protein.

An advantage of the invention is the ability to increase heterologous protein production and direct amino acid substitution to a desired codon position using a nonsense codon and producing the protein in a suppressor mutant such that a known amino acid is substituted in each suppressor host. Stabilization of the mRNA transcript by inhibiting the nonsense-mediated mRNA decay pathway increases the half-life of the transcript (decreases its decay rate) thereby allowing increased translation from the transcript. Preferably the nonsense codon is present in a transcript destabilizing 5′ portion of the transcript. Preferably the transcript containing the nonsense codon decays rapidly in the presence of an unaltered wild-type nonsense-mediated mRNA decay pathway, and decays at least 2 fold more slowly in the presence of a nonsense-mediated mRNA decay pathway inhibited by the method of the invention.

By "substantially pure DNA" is meant a DNA that is not immediately contiguous with (i.e., covalently linked to) both of the coding sequences with which it is immediately contiguous (i.e., one at the 5′ end and one at the 3′ end) in the naturally-occurring genome of the

organism from which the DNA of the invention is derived. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote; or which exists as a separate molecule (e.g., a cDNA or a genomic or cDNA fragment produced by PCR (polymerase chain reaction) or restriction endonuclease digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequences.

By "polypeptide" is meant any chain of amino acids, regardless of length or post-translational modification (e.g., glycosylation).

By "inhibited nonsense-mediated mRNA decay pathway" is meant decreased turnover of a nonsense codon-containing mRNA transcript in which the half-life of the nonsense codon-containing mRNA is at least 2 fold greater in a nonsense-mediated mRNA decay pathway altered by the methods of the invention relative to its half-life in a wild type cell. Techniques for measuring mRNA half-life are described herein and in Parker R. et al. (1991) Meth. Enzymol. 194:415-423.

By "transcript destabilizing 5′ portion" is meant a 5′ proximal region of an mRNA transcript in which region the presence of a nonsense codon results in an increased rate of transcript degradation by at least 2 fold compared to the normal transcript in a wild-type organism. Determination of a transcript destabilizing 5′ portion is readily performed by one of ordinary skill in the art. The DNA sequence is altered at each of at least three known positions in separate copies of the same DNA to encode a nonsense codon at each position. The half-life the transcript from each altered DNA is compared to the wild-type transcript by standard techniques. An

approximately 2 fold or more decrease in half-life for the altered transcript in a cell expressing wild-type nonsense-mediated mRNA decay pathway activity indicates that the nonsense codon is in a transcript destabilizing region. The region 5′ proximal of the most downstream destabilizing nonsense codon position is considered a transcript destabilizing 5′ portion.

By "Nmd2p" is meant the protein (SEQ ID NO:2) encoded by the *Saccharomyces cerevisiae* gene, *NMD2* (SEQ ID NO:1), which is involved in the nonsense-mediated mRNA decay pathway.

By "Upf1p" is meant the protein encoded by the *Saccharomyces cerevisiae* gene, *UPF1*, which is involved in the nonsense-mediated mRNA decay pathway (Leeds, P. et al. (1992), supra).

By "substantially pure polypeptide" is meant that the nonsense-mediated mRNA decay polypeptide or fragment thereof provided by the invention is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, nonsense-mediated mRNA decay polypeptide or fragment. A substantially pure nonsense-mediated mRNA decay polypeptide or fragment thereof is obtained, for example, by extraction from a natural source; by expression of a recombinant nucleic acid encoding a nonsense-mediated mRNA decay polypeptide or fragment thereof; or by chemically synthesizing the polypeptide or fragment. Purity can be measured by any appropriate method, e.g., column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

By "carboxy terminal fragment (SEQ ID NO:4) of Nmd2p" is meant the sequence including amino acid 326 to amino acid 1089 (SEQ ID NO:4) or a fragment thereof. The

carboxyl terminus is any polypeptide including SEQ ID NO:4 or a fragment thereof that substantially inhibits nonsense-mediated mRNA decay in a cell when the fragment is expressed above endogenous level.

By "substantially inhibit nonsense-mediated mRNA decay" is meant to cause an increase by at least 2 fold in the half-life of an mRNA of interest in the presence of an inhibiting agent (e.g., a chemical agent, a polypeptide fragment, or like substance) that interferes with the functioning of the proteins of the nonsense-mediated mRNA pathway.

By "overexpressed polypeptide" is meant the *in vivo* expression of a DNA sequence to produce a polypeptide in a quantity at least 2 fold greater than the quantity of the same polypeptide expressed from the endogenous transcription /translation regulatory elements of the DNA sequence of interest. In the case of the expression of a gene fragment, the endogenous regulatory elements are those of the native gene.

By "substantially increased transcript stability" is meant an increase in the half-life of an mRNA transcript by at least 2 fold in the presence of an inhibited nonsense-mediated mRNA decay pathway. The half-life of an mRNA transcript can be measured by extracting at various time points total mRNA from a cell expressing the gene of interest. This is followed by determining the abundance of a transcript over time by Northern analysis using a labelled (e.g., radiolabelled probe) nucleic acid probe to visualize the transcript. Increased transcript stability can also be inferred from increased expression of a polypeptide from the gene of interest in the presence of an inhibited nonsense-mediated mRNA pathway.

By "essentially no functional protein produced" is meant sufficient lack of a particular protein (e.g.,

Nmd2p or Upf1p) in a cell such that the nonsense-mediated mRNA decay pathway is sufficiently inhibited to result in a substantial increase in the stability of mRNA transcripts containing a nonsense codon in a transcript destabilizing 5' portion such as is described herein for the *PGK1* transcript.

By "operably linked" is meant that a gene and a regulatory sequence(s) are connected in such a way as to permit gene expression when the appropriate molecules (e.g., transcriptional activator proteins) are bound to the regulatory sequence(s).

By "inducible regulatory sequences" is meant regulatory sequences (e.g., transcriptional regulatory sequences) whose function is initiated by the introduction of one or more external agents to the cell culture medium and whose function is inhibited by the removal of the external agents.

By "sense transcript" is meant the transcript resulting from expression of the gene-encoding DNA strand from operably linked regulatory sequences. By "antisense transcript" is meant the transcript resulting from expression of the strand complementary to the sense strand from operably linked regulatory sequences. The antisense transcript binds to and inhibits translation of the sense transcript.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described below. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the detailed description, and from the claims.

Detailed Description

The drawings will first be described.

Drawings

Fig. 1 is a photograph of yeast colonies on X-Gal medium and a diagram identifying the fusion plasmids contained in the yeast strains for a two-hybrid screening assay. β-galactosidase activity indicates interaction between NMD2 and UPF1 fusion products.

Fig. 2 is the DNA sequence (SEQ ID NO:1) and the deduced amino acid sequence (SEQ ID NO:2) of NMD2. Cloning of the *NMD2* gene and determination of its DNA sequence are described herein. The predicted amino acid sequence is indicated in single-letter code and shown below each line of DNA sequence. Position number 1 corresponds to the A of the ATG initiation codon. The *NMD2* open reading frame is interrupted by an intron of 113 nucleotides in which the conserved 5′ splice site [GUAUGU], branchpoint [UACUAAC], and 3′ splice site [AG] are underlined. Transcription initiation sites at nucleotides -56, -60, -64, and -67 (relative to the initiator ATG) were determined by primer extension analysis and are indicated by vertical arrows. The putative TATA box and Abf1p binding consensus sequence, located between positions -219 to -213 and -198 to -186 in the *NMD2* promoter region are respectively underlined by dashed lines. Double underlined residues fit the consensus for a bipartite nuclear localization signal (Dingwall and Laskey, (1991) Trends Biochem. Sci. 16:478-481). The positions where FLAG- or MYC-epitope tag sequences were inserted are indicated by lollipops and the position where the original *GAL4-NMD2* fusion begins

is indicated by an arrow with a right angle stem. The bent arrow also indicates the start of the DNA sequence from nucleotide 1089 to nucleotide 3383 (SEQ ID NO:3) encoding the carboxyl terminal amino acid sequence from amino acid 326 to amino acid 1089 (SEQ ID NO:4) of Nmd2p, a peptide fragment which, when overexpressed, binds to Upf1p and inhibits the nonsense-mediated mRNA decay pathway.

Figs. 3A to 3C are diagrams of insertion and deletion experiments performed to assess the active regions of *NMD2* gene. DNA fragments associated with *NMD2* function are indicated. Fig. 3A is a restriction map of the *nmd2::HIS3* allele. Fig. 3B is a restriction map of the *NMD2* gene. Fig. 3C is a diagram of the results of a complementation analysis to determine functional portions of Nmd2p.

Figs. 4A to 4C are reproductions of autoradiograms. Fig. 4A is reproduced from a Southern analysis of wild type and HIS3-disrupted NMD2 associated with *NMD2* gene disruption. Fig. 4B is reproduced from a Northern analysis of the stability of different nonsense-containing *PGK1* alleles in *NMD2* and *nmd2::HIS3* haploid yeast strains. Fig. 4C is reproduced from a Northern analysis of *CYH2* pre-mRNA and mRNA transcript stability.

Figs. 5A to 5B are reproductions of Northern analysis autoradiograms which record the *CYH2* transcript stability phenotypes associated with disruption of both the *NMD2* and *UPF1* genes or overexpression of Nmd2p fragments.

This invention relates to a DNA sequence, a protein, and methods useful in inhibiting the nonsense-mediated mRNA decay pathway in a cell, preferably in a yeast cell, thereby stabilizing an mRNA transcript which contains a nonsense codon. Preferably, the nonsense

codon is in a transcript destabilizing 5′ portion of the transcript. Stabilization of the transcript allows increased translation and increased production of a heterologous protein of interest. The protein of interest can be a full length protein if the nonsense codon is suppressed. The protein of interest can be a desired N-terminal fragment of a protein if the nonsense codon is not suppressed.

Examples

The current invention is illustrated by the following examples, which are not to be construed as limiting in any way. The examples illustrate the invention by describing the *NMD2* gene, the Nmd2 protein, and its C-terminal fragment. Methods of substantially inhibiting the nonsense-mediated mRNA decay pathway in a cell, and methods of producing heterologous proteins and fragments of proteins are also described. These methods inhibit the nonsense-mediated mRNA decay to increase transcript stability.

Example 1: Identification of a Gene Encoding a Putative Upf1p-interacting Protein

To identify a gene or genes encoding putative Upf1p-interacting proteins, the yeast two-hybrid system was used. This method of detecting protein-protein interactions in yeast is based on the observation that the DNA binding and transcriptional activation functions of the *GAL4* protein (Gal4p) can reside on two distinct chimeric polypeptides and still activate transcription from a *GAL* UAS, provided that the two polypeptides can interact with each other (Fields and Song, (1989) Nature 340:245-246; Chien, C.-T. et al., (1991) Proc. Natl. Acad. Sci. 88:9578-9582). As employed herein, the first hybrid was cloned into a plasmid (such as pMA424; (Ma, J. and Ptashne, M. (1988) Cell 55:443-446) in which the

entire *UPF1* coding region was fused in-frame to the Gal4p DNA binding domain (amino acids 1-147 of Gal4p). Construction of plasmid pMA424-*UPF1* was performed by a three-fragment ligation. A fragment of 144 bp from the initial ATG codon to the 48th codon of *UPF1* was amplified by the polymerase chain reaction (PCR) using UPF1-TH-5′ (SEQ ID NO:5) and UPF1-TH-3′ (SEQ ID NO:6) as oligonucleotide primers (Table 1).

TABLE 1 - Oligonucleotide Primers

UPF-TH-5′	5′-CCGGAATTCATGGTCGGTTCCGGTTCT-3′	(SEQ ID NO:5)
UPF-TH-3′	5′-AGTGACTTGAGCCTC-3′	(SEQ ID NO:6)

Amplification with these primers led to the introduction of an *Eco*RI site adjacent to the initiator ATG. The PCR-amplified fragment was digested with *Eco*RI and *Bst*XI and ligated with a *Bst*XI-*Bam*HI fragment (including the rest of the *UPF1* coding region and approximately 1 kb 3′ distal to the translational termination site including the entire 3′UTR) into plasmid pMA424 digested by *Eco*RI and *Bam*HI. DNA sequence analysis confirmed the primary structure of the construct.

Second hybrids were encoded by *S. cerevisiae* genomic DNA libraries in plasmids pGAD(1-3) (Chien, C.-T. et al. (1991) Proc. Natl. Acad. Sci USA 88:9578-9582) fused, in the three reading frames, to sequences encoding the Gal4p transcriptional activation domain (amino acids 768-881). Both were cotransformed into a *Saccharomyces cerevisiae* strain that contained an integrated *GAL1-LacZ* reporter construct (such as the *S. cerevisiae* strain GGY1::171 (*Δgal4 Δgal80 URA3::GAL1-LacZ his3 leu2*)) (Gill, G. and Ptashne, M. (1987) Cell 51:121-126) or

equivalent strain well known to those of ordinary skill in the art of yeast genetics.

In performing the two-hybrid screening method, the GGY1::171 yeast strain was cotransformed with both pMA424-*UPF1* and a library containing genomic DNA fragments fused to the *GAL4* activation domain. After 3-4 days of growth on SD-His-Leu plates at 30°C, His^+Leu^+ transformants were replica-plated to SSX plates and were incubated until blue colonies appeared as described in Rose, M.D. et al. (1990) Methods in Yeast Genetics: A Laboratory Course Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY). False positive colonies due to cloning of the GAL4 gene into the pGAD vectors were eliminated by PCR yeast cellular DNA using the *GAL4*-specific primers GAL4-5′ (from nucleotide 1206 to 1229 of the *GAL4* gene) and GAL4-3′ (from nucleotide 2552 to 2528 of the *GAL4* gene)(Laughon and Gesteland, (1984) Mol. Cell Biol. 4:260-267). Cells from the remaining blue colonies were grown in SD-Leu medium and plasmids were recovered and transformed into the *E. coli* strain MH6 by electroporation. The activation domain (pGAD) plasmids from the library were identified by their ability to complement an *E. coli leuB* mutation due to the presence of the plasmid-borne *LEU2* gene. According to the two-hybrid test, transcriptional activation depends interaction between the *UPF1* fusion product and the test fragment fusion product. To confirm that transcriptional activation was dependent on the presence of both gene fusions, the isolated library plasmids were retransformed into the original GGY1::171 strain with either: 1) pMA424-*UPF1*, a *GAL4* DNA-binding domain-*UPF1* fusion plasmid; 2) pMA424, the *GAL4* DNA binding domain vector only; 3) pMA424-*CEP1*, a *GAL4* DNA-binding domain-*CEP1* fusion plasmid; or 4) pMA424-*LAM5*, a *GAL4* DNA-binding domain-*LAM5* fusion plasmid, where *CEP1* and *LAM5* genes are

negative control genes whose gene products are known not to bind to *UPF1* gene product. Plasmids that yielded blue colonies only with the pMA424-*UPF1* fusion were characterized further by restriction mapping, Southern analysis, and sequence analysis (see e.g., Sambrook et al., (1989), Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York). DNA sequences were compared to existing sequence databases using the FASTA program (Devereux, J. et al., (1984) Nucleic Acids Res. 12:387-395). Colonies expressing detectable β-galactosidase activity were sought by screening approximately 400,000 transformants.

Eighty-seven colonies that demonstrated β-galactosidase activity (i.e., colonies pale blue to dark blue on X-Gal plates) on the initial screen were isolated. Because the libraries were constructed using genomic DNA from a *GAL4* wild-type strain, plasmids containing the *GAL4* gene, or fragments thereof, are capable of activating transcription of the *GAL1-LacZ* reporter gene. These false positive colonies were eliminated by use of the polymerase chain reaction (PCR; White, T.J. et al., (1989) Trends Genet. 5:185-189) with *GAL4* specific primers. The library plasmids from the remaining colonies were rescued and tested for specificity by retransforming them into the original strain with either: 1) the *GAL4-UPF1* fusion; 2) the *GAL4* DNA binding domain vector only; 3) an unrelated fusion, *GAL4-CEP1*; or 4) an unrelated fusion, *GAL4-LAM5* (Bartel, P. et al., (1993) Biotechniques 14:920-924). Forty-two plasmids that yielded blue colonies only with *GAL4-UPF1* fusion plasmid-containing strains were characterized further by restriction mapping, Southern analysis, and partial DNA sequence analysis using standard techniques (see e.g., Sambrook, J. et al., (1989) supra.

Fig. 1 shows the blue colony formation that occurred only when *NMD2* and UPF1 fusion plasmids were present in the same host strain. The *S. cerevisiae* tester strain GGY1::171 was co-transformed with the original library isolate pGAD2-*NMD2* and one of the following plasmids: 1) pMA424-*UPF1*, 2) pMA4242, 3) pMA424-*CEP1*, or 4) pMA424-*LAM5* (pMA424-*CEP1* was obtained from Richard Baker of the University of Massachusetts Medical Center, Worcester, MA; pMA424-*LAM5* was obtained from Stanley Fields and Paul Bartel of State University of New York, Stony Brook, N.Y. Individual Leu^+ His^+ transformants were selected and streaked on synthetic medium plates lacking histidine and leucine. β-galactosidase activity assays were performed by replica-plating the transformants onto SSX plates containing X-Gal. Cells were incubated at 30°C for 24-48 hours for development of blue color.

Southern blot analysis of the isolated plasmids was performed by first extracting total yeast genomic DNA according to the method of Holm, C. et al. (1986) Gene 42:169-173. After restriction digestion, DNA was electrophoresed on 0.8% agarose gels, transferred and cross-linked to Zetaprobe membranes (BioRad, Richmond, CA) as described in Sambrook, J. et al. (1989), supra. Filters were prehybridized 2-3 hours at 42°C in 5X SSPE, 40% formamide, 5X Denhardt's solution, 0.1% SDS, and 4 mg/ml salmon sperm DNA. A radiolabeled NMD2 probe (1.2 kb *Cla*I-*Eco*RI fragment), generated by random priming, was added and filters were hybridized overnight at 42°C. Filters were washed twice in 1X SSC, 0.1% SDS at room temperature and once in 0.1X SSC, 0.1% SDS at 58°C before analyzing on a Betagen Blot Analyzer (Herrick, D. et al., (1991) Mol. Cell. Biol. 10:2269-2284).

DNA sequences were determined by the method of Sanger, F. et al., (1978) Proc. Natl. Acad. Sci. 74:5463-

5467. Overlapping fragments of the NMD2 gene were subcloned in Bluescript and sequenced by annealing oligonucleotide primers specific to the T3 or T7 promoter regions of the plasmid or by using oligonucleotide primers which annealed within the subcloned inserts.

Nine different genes were isolated by the following procedure. An *S. cerevisiae* genomic DNA library of Sau3A partial fragments constructed in YCp50 was used (Rose, M. et al. (1987) Gene 60:237-243). Colony hybridization was performed as described in Sambrook, J. et al., (1989), supra, using the same conditions described for the genomic DNA Southern hybridization. Approximately three genomic equivalents were screened. Disruption of the *NMD2* gene was performed by transforming the diploid strain W303 (*MATa/MATα ade2-1/ade2-1 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1 ura3-1/ura3-1 can1-100/can1-100*) with a *Sac*I-*Sal*I fragment from Bs-*nmd2*::*HIS3* and selecting His$^+$ transformants (the *Sac*I and *Sal*I sites are in the polylinker of the Bluescript KS$^+$ cloning vector, Stratagene, La Jolla, CA; Rothstein, R. (1991) "Targeting, disruption, replacement, and allele rescue: integrative DNA transformation in yeast.", in Methods in Enzymology 194: Guide to Yeast Genetics and Molecular Biology, C. Guthrie and G. Fink, eds., Academic Press, pp. 281-301; Thomas, B.J. and Rothstein, R. (1989) Cell 56:619-630). The disruption event was confirmed by Southern analysis. Sporulation and tetrad analysis yielded haploid strains containing *nmd2*::*HIS3* disruptions.

Six of the isolated genes encoded putative Upf1p-interacting proteins because their activity in the assay was dependent on fusion to the *GAL4* activation domain. The remaining three genes did not require the presence of the *GAL4* activation domain, were likely to

possess their own activation domains and nuclear localization signals and were not examined further.

Six genes were found to encode putative Upf1p-interacting proteins; two genes are identical to previously characterized yeast genes, i.e., *DBP2*, a gene encoding a putative RNA helicase with homology to the mammalian p68 RNA helicase (Iggo, R. D. et al., (1991) Mol. Cell. Biol. 11:1326-1333). The other four have no apparent homologues in the available data bases. One of the genes, herein named *NMD2*, is characterized herein, and its uses for the production of heterologous proteins in yeast are disclosed.

Example 2: Molecular Cloning of the *NMD2* Gene

As defined by a qualitative β-galactosidase assay, Nmd2p showed a specific dependency on Upf1p in the two-hybrid system. Cells expressing a *GAL4* activation domain-*NMD2* fusion demonstrated strong β-galactosidase activity when simultaneously expressing a *GAL4* DNA-binding domain-*UPF1* fusion, but had no detectable β-galactosidase activity when co-transformed with plasmids encoding only the *GAL4* DNA-binding domain-*LAM5* fusion (Fig. 1). Further evidence for the specificity of the interaction(s) was obtained by analyzing the effects of specific deletions within the *UPF1* portion of the *GAL4* DNA-binding domain-*UPF1* fusion. Deletions in all but one segment of the *UPF1* coding region eliminated Nmd2p-Upf1p interaction in the two-hybrid assay.

The *GAL4* activation domain-*NMD2* plasmid recovered in the two hybrid screen contained only a fragment of the *NMD2* gene. To isolate the entire gene, a 1.2 kb *Cla*I-*Eco*RI fragment downstream of the *GAL4* activation domain in the fusion plasmid was used to screen a yeast YCp50 genomic DNA library (Rose, M. et al., (1987) supra). Two independent clones with identical restriction patterns

were isolated. By restriction mapping, Southern analysis, and subsequent testing for complementation of an *NMD2* chromosomal deletion, the *NMD2* gene was localized to a 5.2 kb *Xba*I-*Sal*I DNA fragment as shown in Figs. 3A to 3C.

A restriction map of the *nmd2::HIS3* allele is shown in Fig. 3A. The *Xba*I-*Cla*I fragment of the *NMD2* gene, was deleted and replaced with the yeast *HIS3* gene. The left arrow in Fig. 3A represents the *HIS3* gene and indicates the direction of transcription. The right arrow of Fig. 3A represents the *NMD2* open reading frame.

A restriction map of the NMD2 gene is shown in Fig. 3B. The *NMD2* open reading frame and direction of transcription are indicated by an open arrow interrupted by a stippled box that indicates the position of the intron. The box labeled probe indicates the DNA fragment used for screening the genomic DNA library. In Figs. 3A and 3B, the black box represents a segment from the cloning vector YCp50 and the restriction site abbreviations are: B, *Bam*HI; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; P, *Pst*I; S, *Sal*I; Xb, *Xba*I.

To determine the regions of Nmd2p required for complementation of a disrupted nonsense mediated mRNA pathway in a *nmd2::HIS3* strain, deletion experiments were performed. In Fig. 3C, lines represent DNA fragments which were subcloned into an appropriate vector (such as pRS315). These constructs were transformed into the yeast strain HFY1300, or equivalent, which contains a partial chromosomal deletion of *NMD2* and lacks nonsense-mediated mRNA decay activity (see also, Figs. 4A and 4B). Total RNA was isolated from these transformants and Northern analysis was performed using a radiolabeled probe derived from the *CYH2* gene (He, F. et al., (1993) Proc. Natl. Acad. Sci. 90:7034-7039). Complementing activity was scored by measuring the relative abundance

of the *CYH2* pre-mRNA and mRNA in each strain. (+) and (-) indicate the ability or inability, respectively, to complement the *NMD2* chromosomal deletion, i.e., to restore the *CYH2* pre-mRNA to the marginally detectable levels characteristic of wild-type cells (He, F. et al., (1993) Proc. Natl. Acad. Sci. 90:7034-7039).

To obtain a physical map position for the *NMD2* gene, the 1.7 kb *Xba*I-*Cla*I fragment was used to probe PrimeClone blots (American Type Culture Collection, Rockville, MD) containing characterized fragments of most of the *S. cerevisiae* genome (ATCC accession number 7155) known to lie on the right arm of chromosome VIII (Riles, L. et al., (1993) Genetics 134:81-150). This fragment is located between the *put*2 and *CUP*1 loci at a map position approximately 260 kb from the left telomere (Riles et al., (1993 supra).

Example 3: Determining the Primary Sequence of the *NMD2* Gene

The complete sequence of the *NMD2* gene was determined (SEQ ID NO:1). The *NMD2* coding region is 3267 nucleotides in length, encoding an acidic (predicted pI = 4.8) protein of 1089 amino acid residues (SEQ ID NO:2) with a predicted molecular weight of 127 kD. This interpretation of the *NMD2* sequence relies on the prediction of a 113-nucleotide intervening sequence that commences at position +7 and divides the gene into two exons (Fig. 2).

Four observations support the existence of this intron. First, the sequence contains all three of the standard consensus sequences expected of an intron (5′ splice site [GUAUGU], branchpoint [UACUAAC], and 3′ splice site [AG]) (Fig. 2). Second, as is true for most introns in yeast (Fink, G.R. (1987) Cell 49:5-6), this intron is located at the 5′ end of the NMD2 gene (six

nucleotides downstream from the predicted initiator ATG; Fig. 2). Third, specific primer extension products were detected by using two different oligonucleotide primers complementary to mRNA sequences downstream of the predicted 3′ splice site, but not by using a primer complementary to sequences within the intron. Finally, using the FLAG or c-*MYC* epitope tags (Hopp, T.P. et al., (1988) Biotechnology 6:1204-1210; Prickett et al., (1989); Evan, G.I. et al., (1985) Mol. Cell. Biol. 5:3610-3616) and epitope-specific monoclonal antibodies, the expression of a 127 kD polypeptide was detected when the FLAG or c-*MYC* sequences were inserted adjacent to the putative initiator ATG (FLAG-2-*NMD2* or c-*MYC*-*NMD2* alleles), but not when the FLAG sequence was inserted adjacent to the second ATG (FLAG-1-NMD2 allele). The second ATG is located within the putative intron, 37 nucleotides downstream of the predicted intron branchpoint, and is in frame with the major downstream open reading frame but not with the first ATG. It is important to note that both the FLAG-1-*NMD2* and FLAG-2-*NMD2* alleles are functional in that they both show wild-type ability to complement a chromosomal deletion of *NMD2* (Fig. 3C). These results indicate that the FLAG-1 sequence inserted downstream of the second ATG has been removed by splicing out of the putative intron in the *NMD2* gene.

Analysis of the *NMD2* transcript was consistent with the predicted open reading frame. Northern analysis of total cellular RNA, using the *NMD2 Xba*I-*Cla*I fragment as a probe, identified a transcript of approximately 3.6 kb in size. Multiple transcription initiation sites were mapped to positions -56, -60, -64, and -67 using primer extension analysis (see e.g., Boorstein, W.R. and Craig, E.A. (1989) Meth. Enzymol. 180:347-369). A putative TATA box, required for most RNA polymerase II transcription

(Struhl, K. (1987) Cell. 49:295-297), lies at positions -219 to -213 in the *NMD2* promoter region and another regulatory element, an Abf1p binding consensus sequence (Della Seta, F. et al., (1990) J. Biol. Chem. 265:15168-15175), is located within positions -198 to -186 (Fig. 2).

Structural features of the *NMD2* protein (Nmd2p; SEQ ID NO:2) inferred from the sequence analysis include a highly acidic internal fragment (36.8% aspartic acid and 25.6% glutamic acid) from residues 843 to 975 near the C-terminus and a possible bipartite nuclear localization signal at the N-terminus of the protein (i.e., within residues 26 to 29 and 42 to 46) (Fig. 2; Dingwall and Laskey, (1991) supra). Comparison of the Nmd2p sequence with those in the Swissprot and Pir protein sequence databases using the FASTA or TFASTA comparison programs (Devereux et al., (1984) supra) did not reveal any extensive identity with known protein sequences. However, three domains of Nmd2p have substantial similarity to regions of other proteins. The first domain, spanning Nmd2p amino acids 1 to 390, has 17.7% sequence identity and 47% similarity with translational elongation factor 2 (Eft1p and Eft2p) from *S. cerevisiae* (Perentesis, J.P. et al., (1992) J. Biol. Chem. 267:1190-1197). The second domain, from amino acids 400 to 810 in Nmd2p, shares 19.5% sequence identity and 42.6% similarity with the *S. cerevisiae* mitochondrial RNase P protein Rpm2p (Dang, Y. and Martin, N.C. (1993) J. Biol. Chem. 268:19791-19796). The third domain, encompassing the acidic stretch from amino acids 820 to 940, has 34% sequence identity and 63.2% similarity with human and mouse nucleoproteins (Lapeyre, B. et al., (1987) Proc. Natl. Acad. Sci. 84:1472-1476; Bourbon, H-M et al., (1988) J. Mol. Biol. 200:27-638) and 34% identity and 65% similarity to the mammalian polymerase I

transcriptional factors hUBF and mUBF (Jantzen, H-M et al., (1990) Nature 344:830-836; Hisatake, K. et al., (1991) Nucleic Acids Res. 19:4631-4637). In hUBF and mUBF this domain has been shown to be important for interaction with other proteins (Jantzen et al., (1990) supra) and, as described below, is also true for Nmd2p.

Example 4: *NMD2* Disruption Does Not Affect Cell Viability and Selectively Stabilizes Nonsense-containing mRNAs

A *NMD2* gene disruption experiment was performed to assess the cellular requirement for Nmd2p. The *nmd2::HIS3* disruption described in Fig. 3A was constructed. Plasmid Bs-*nmd2::HIS3* encodes the same *NMD2* disruption and contains a 0.6 kb *Cla*I-*Xba*I fragment in the 5'-end of *NMD2*, a 1.7 kb *Xba*I-*Cla*I fragment of *HIS3* and a 1.2 kb *Cla*I-*Eco*RI fragment in the *NMD2* coding region in Bluescript. A *Sac*I-*Sal*I fragment carrying the *nmd2::HIS3* allele was isolated from plasmid Bs-*nmd2::HIS3* and used to transform the yeast diploid strain W303 for homologous recombination into one of the *NMD2* alleles. His^+ transformants were sporulated and tetrads were individually dissected. Four viable spores were obtained from each tetrad analyzed. Genomic DNAs from parental diploid and progeny haploid strains were isolated, digested with *Eco*RI. Confirmation of integration is shown by the Southern analysis of Fig. 4A in which lane P1 contains DNA isolated from the homozygous *NMD2/NMD2* diploid strain W303; lane P2 contains DNA isolated from a diploid *nmd2::HIS3/NMD2* His^+ transformant of W303 (HFY1000); and lanes 1A to 1D contain DNA isolated from the progeny of four viable spores dissected from the same tetrad represent the wild-type and disrupted alleles of *NMD2*, respectively. Other bands in the figure are not specific to *NMD2*.

Haploid strains containing the *nmd2::HIS3* disruption were compared to isogenic *NMD2* strains for their ability to grow on different carbon sources (glucose, galactose, and glycerol) at temperatures ranging from 18°C to 37°C and no differences in growth rates were detected between mutant and wild-type strains. These data indicate that *NMD2* is non-essential for cell viability. Since disruption of the *NMD2* gene was not lethal, the activities of the nonsense-mediated mRNA decay pathway in both *NMD2* and *nmd2::HIS3* strains were compared.

The following method was used to analyze transcript stability in strains having an *NMD2* disruption, and is useful to one of ordinary skill in the art for analyzing the stability of any transcript of interest. Yeast centromere plasmids carrying six different *PGK1* nonsense alleles were constructed previously (Peltz, S.W. et al., (1993) supra). These plasmids were transformed into *NMD2* and *nmd2::HIS3* strains and the abundance of *PGK1* nonsense-containing mRNAs was assessed by Northern analysis as shown in Fig. 4B. Disruption of the *NMD2* gene stabilizes *PGK1* mRNAs containing early nonsense mutations. Isogenic *NMD2* and *nmd2::HIS3* haploid yeast strains harboring different nonsense-containing *PGK1* alleles (HFY1201 to HFY1206 and HFY1301 to HFY1306) were constructed by transforming HFY1200 and HFY1300 with each of the six plasmids harboring the nonsense-containing *PGK1* alleles described previously (Peltz, S.W. et al., (1993) Genes & Devel 7:1737-1754).

Total RNA was isolated from these strains and analyzed by Northern blotting using a radiolabeled oligonucleotide probe complementary to the tag sequence located in the 3′ untranslated region of *PGK1* nonsense-containing mRNAs (Peltz, S.W. et al., (1993) Genes &

Devel 7:1737-1754). The location of the nonsense mutation in each *PGK1* transcript is presented as a percentage of the *PGK1* protein-coding region that is translated before the mutation is encountered (Peltz, S.W. et al., (1993) Genes & Devel 7:1734-1754).

Decay rates of mRNA were measured as previously described (Herrick et al., (1990) supra; Parker, R. et al., (1991) Meth. Enzymol. 194:415-423; Peltz, S.W. et al., (1993) supra). For measurement of mRNA abundance, yeast cells (20 ml) were grown to OD_{600}=0.5-0.7 at 24°C for 30 min. An aliquot (2 ml) of concentrated cell culture was collected and frozen quickly on dry ice. Total yeast RNA was isolated as described previously (Herrick et al., (1991) supra). For both decay rate measurements and abundance measurements equal amounts (usually 20 μg) of total RNA from each sample were analyzed by Northern blotting, generally using probes labeled in random priming reactions (see, e.g., Sambrook, J. et al., (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). Hybridization conditions for such blots were as described for genomic Southern hybridization. When oligonucleotide probes were used, the hybridization conditions were those described by Peltz, S.W. et al. (1993) supra. Northern blots were quantitated with a Betagen Blot Analyzer (Herrick et al., (1990) supra).

Nonsense mutations in the 5′ two-thirds of the *PGK1* coding region reduced the abundance of the corresponding mRNAs 5- to 20-fold (Peltz, S.W. et al., (1993) supra). The abundance of *PGK1* mRNAs with nonsense mutations in the downstream third of the coding region is unaffected. Disruption of the *NMD2* gene restored wild-type levels to all four of the *PGK1* transcripts normally subject to nonsense-mediated mRNA decay (Fig. 4B). As a control, the abundance of the wild-type *PGK1* and *ACT1*

mRNAs, and the half-life of the MATα1 mRNA in the same cells, was found to be unaffected by the *nmd2::HIS3* disruption.

Northern analysis was also used to measure the relative abundance of the *CYH2*, *RP51B*, and *MER2* pre-mRNAs in *NMD2*. As shown in Fig. 4C, decay rates of *CYH2* pre-mRNA and mRNA were determined by Northern analysis of RNAs isolated at different time points after transcription was inhibited by shifting cultures of isogenic *NMD2* (HFY2206) and *nmd2* (HFY2106) strains to 36°C. Samples were taken for 36 min and the blot was hybridized with a radiolabeled *CYH2* DNA probe. To construct strains HFY2206 and HFY2106, stain HFY2000 was produced by integrative transformation; selected and tested to contain the temperature sensitive *rpb*1-1 allele. Strain HFY2000 was transformed with pRS315 (or similar yeast shuttle plasmid; (Sikorski and Hieter, (1989) Genetics 122:19-27) or pRS315-*NMD2*(X-S) (containing a 5.2 kb *Xba*I-*Sal*I fragment of *NMD2* in pRS315) and a plasmid harboring a *PGK1* allele with a nonsense mutation at the *Bgl*II site (Peltz, S.W. et al., (1993) Genes & Devel 7:1737-1754). The abundance of the inefficiently spliced *CYH2* and *RP51B* pre-mRNAs, and the *MER2* pre-mRNA (whose splicing is regulated by *MER1*; Engebracht et al., 1991) was markedly increased in strains carrying the *nmd2::HIS3* disruption. Disruption of the *NMD2* gene reduces the decay rate of the *CYH2* pre-mRNA approximately four-fold, i.e., from a half-life of 1.5 min to a half-life of 6.0 min without a concomitant effect on the half-life of the *CYH2* mRNA (Fig. 4C). These results are equivalent to those obtained in *UPF1* knockout strains (He et al., (1993) supra) indicating that Nmd2p is a Upf1p-interacting protein and that *NMD2* is a novel component of the nonsense-mediated mRNA decay pathway.

Example 5: Overexpression of Truncated Nmd2p in the Cytoplasm Results in a Dominant-negative Nonsense-mediated mRNA Decay Phenotype

The region of Nmd2p that interacts with Upf1p was determined by generating 5′ and 3′ deletions of the original *NMD2* fragment, fusing them in-frame to the *GAL4* activation domain, and assaying the resultant constructs for interaction with Upf1p using the two-hybrid system. Fusions encoding either 237 or 477 amino acids from the amino-terminus of the original fragment demonstrated no detectable β-galactosidase activity. However, fusions encoding either 526 or 286 amino acids from the carboxyl-terminus of the original fragment did demonstrate detectable β-galactosidase activity. These results indicate that the acidic C-terminal domain of Nmd2p interacts with Upf1p.

The identification of Nmd2p as a Upf1p-interacting protein in a two-hybrid screen and the observation that disruption of the *NMD2* gene yielded a nonsense-mediated mRNA decay phenotype equivalent to that obtained in strains harboring *upf1* mutations suggests that Upf1p and Nmd2p interact with each other *in vivo* and that they perform different functions in the same decay pathway. This conclusion is strengthened by the finding that double mutants in which both the *UPF1* and *NMD2* gene products are functionally absent produce strains that have essentially identical phenotypes with regard to the half-lives of test mRNA transcripts such as *CYH2* pre-mRNA. Thus, Upf1p and Nmd2p must function in closely related steps of the nonsense-mediated mRNA decay pathway.

A truncated form of Nmd2p was expressed in both the nucleus and cytoplasm and activity was functionally localized within the cell to the cytoplasm. The original *GAL4* activation domain-*NMD2* fusion plasmid encodes 764 amino acids of the C-terminal segment of Nmd2p (SEQ ID

NO:4). Transcription of this *GAL4*-activation domain-*NMD2* fusion was driven by a cryptic promoter in the *ADH1* terminator present in the vector and the fusion protein was targeted to the nucleus by the SV40 T antigen nuclear localization signal (Chien, C-T. et al., (1991) supra. The 6.0 kb *Hin*dIII fragment encoding this fusion protein was also subcloned into pGAD2F so that transcription of the fusion protein was driven by the more potent *ADH1* promoter. Since the SV40 T antigen nuclear localization signal (NLS) of the fusion protein is in a 36 bp *Eco*RI fragment (Benton, B.M. et al., (1990) Mol. Cell. Biol. 10:353-360, we also generated deletions of the NLS in the respective constructs. Plasmids expressing the different fusion proteins were transformed into the haploid strain HFY1200 which is wild-type for both UPF1 and NMD2. HFY1200 was derived from W303 by standard techniques (see, e.g., Rothstein, R. (1991) "Targeting, disruption, replacement, and allele rescue: integrative DNA transformation in yeast.", in Methods in Enzymology 194: Guide to Yeast Genetics and Molecular Biology, C. Guthrie and G. Fink, eds., Academic Press, pp. 281-301). Control experiments, using the two hybrid assay, showed that when *NMD2* plasmids lacking the T antigen NLS were co-transformed with the original plasmid encoding the *GAL4* DNA binding domain-*UPF1* fusion no β-galactosidase activity was detectable, i.e., nuclear localization had been eliminated. Total RNA was isolated from transformants and Northern analysis was performed using a fragment of the *CYH2* gene as a probe.

The Northern analysis results depicted in Fig. 5A show that a double mutant containing both *upf1::URA3* and *nmd2::HIS3* disruptions is phenotypically identical to either *upf1* or *nmd2* single mutants since the *CYH2* pre-mRNA is stabilized in cells containing these disruptions. Total RNAs were isolated from each of the following

strains: HFY3002 (*UPF1/NMD2*); HFY3005 (*upf1Δ/NMD2*); HFY3008 (*UPF1/nmd2Δ*) and HFY3001 (*upf1Δ/nmd2Δ*) (see Table 2). RNAs were analyzed by Northern blotting using a radiolabeled *CYH2* fragment as probe.

TABLE 2 - Yeast Strains

STRAIN	GENOTYPE
HFY1000	*MATa/MATα ade2-1/ade2-1 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1 ura3-1/ura3-1 can1-100/can1-100 nmd2::HIS3/NMD2*
HFY1100	*MATα ade2-1 his3-11,15 leu2-3,112trp1-1 ura3-1 can1-100 NMD2*
HFY1200	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 NMD2*
HFY1300	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 nmd2::HIS3*
HFY1400	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 nmd2::HIS3*
HFY1201	*Same as HFY1200 but containing [pRIPPGKH2 (3) UAG]*
HFY1202	*Same as HFY1200 but containing [pRIPPGKAsp UAG]*
HFY1203	*Same as HFY1200 but containing [pRIPPGKH2 (2) UAG]*
HFY1204	*Same as HFY1200 but containing [pRIPPGKH2 (1) UAG]*
HFY1205	*Same as HFY1200 but containing [pRIPPGKXba UAG]*
HFY1206	*Same as HFY1200 but containing [pRIPPGKBgl UAG]*
HFY1301	*Same as HFY1300 but containing [pRIPPGKH2 (3) UAG]*
HFY1302	*Same as HFY1300 but containing [pRIPPGKAsp UAG]*
HFY1303	*Same as HFY1300 but containing [pRIPPGKH2 (2) UAG]*
HFY1304	*Same as HFY1300 but containing [pRIPPGKH2 (1) UAG]*
HFY1305	*Same as HFY1300 but containing [pRIPPGKXba UAG]*
HFY1306	*Same as HFY1300 but containing [pRIPPGKBgl UAG]*
HFY2000	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rpb1-1 nmd2::HIS3*
HFY2106	*Same as HFY2000 but containing [pRS315] [pRIPPGKBgl UAG]*
HFY2206	*Same as HFY2000 but containing [pRS315-NMD2 (X-S)] [pRIPPGKBgl UAG]*
HFY3000	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 nmd2::HIS3 upf1::URA3*
HFY3001	*Same as HFY3000 but containing [pRS315] [pRS314]*
HFY3002	*Same as HFY3000 but containing [pRS315-NMD2 (X-S)] [pRS314-UPF1]*
HFY3005	*Same as HFY2000 but containing [pR315- NMD2 (X-S)] [pRS314]*
HFY3008	*Same as HFY2000 but containing [pRS315] [pRS314-UPF1]*

The strains listed in Table 2 were prepared in this study. See Peltz, S.W. et al. (1993), supra, for a description of the "pRIPPGK__" plasmids listed above.

Overexpression of truncated Nmd2p in the cytoplasm results in a dominant-negative nonsense-mediated mRNA decay phenotype as shown in Fig. 5B. The yeast strain HFY1200 which is wild-type for both *UPF1* and *NMD2* was transformed with pGAD2F-*NMD2*-ADHt, pGAD2F-*NMD2*-ADHp, pGAD2F, pGAD2F-*NMD2*-ADHt-ΔNLS, pGAD2F-*NMD2*-ADHp-ΔNLS, respectively (see Table 3). Total RNA was isolated from these transformants and analyzed by Northern blotting using a *CYH2* DNA fragment as probe. Lane 1 contained RNA isolated from HFY1300 (control); RNA in other lanes was from transformants of HFY1200 harboring the following plasmids; lane 2, pGAD2F-*NMD2*-ADHt; lane 3, pGAD2F-*NMD2*-ADHp; lane 4, pGAD2F; lane 5, pGAD2F-*NMD2*-ADHt-ΔNLS; lane 6, pGAD2F-*NMD2*-ADHp-ΔNLS. Overexpression of truncated *NMD2* fusion protein localized to the nucleus had no effect on the accumulation of the *CYH2* pre-mRNA (Fig. 5B, lanes 2 and 3). Expression of the cytoplasmically localized fusion protein caused an accumulation of *CYH2* pre-mRNA in a dosage dependent manner, i.e., expression of the fusion protein from the stronger promoter led to a greater accumulation of the *CYH2* pre-mRNA than expression from the weaker promoter (Fig. 5B, lanes 5 and 6). This result establishes that over-expression of a truncated form of the Nmd2p C-terminus (i.e., containing up to 764 amino acids from the C-terminus (SEQ ID NO:4)) results in inhibition of the nonsense-mediated mRNA decay pathway. Shorter C-terminal fragments of Nmd2p are included in the invention as they are readily obtained by screening for inhibiting activity by the two-hybrid screening method coupled with analysis of heterologous transcript stability in the presence of overexpressed amounts of the fragment in the host strain.

TABLE 3 - Plasmids

PLASMIDS	RELEVANT YEAST SEQUENCES
pGAD2F	*GAL4* activation domain-containing plasmid with 2μ and *LEU2* selection markers (Chien, C.-T. et al. (1991) PNAS 88:9578-9582).
pGAD2F-*NMD2*-ADHp	6.0-kb *HindIII* fragment from pGAD2-*NMD2* replaced the 0.6-kb *HindIII-HindIII* fragment of pGAD2F such that the expression of the *GAL4* activation domain -*NMD2* fusion was driven by the *ADH1* promoter.
5pGAD2F-*NMD2*-ADHt	6.0-kb *HindIII* fragment from pGAD2-*NMD2* replaced the 0.6-kb *HindIII-HindIII* fragment of pGAD2F such that the expression of the *GAL4* activation domain -*NMD2* fusion was driven by the *ADH1* terminator.
pGAD2F-*NMD2*-ADHp-ΔNLS	Same as pGAD2F-*NMD2*-ADHp except that the SV40 nuclear localization signal of the fusion protein was deleted.
pGAD2F-*NMD2*-ADHt-ΔNLS	Same as pGAD2F-*NMD2*-ADHt except that the SV40 nuclear localization signal of the fusion protein was deleted.

Example 6: Expression of *NMD2* Antisense Transcript Inhibits the Nonsense-Mediated mRNA Decay Pathway

Nonsense-mediated mRNA decay pathway function of a host cell (i.e, a prokaryotic or eukaryotic cell such as a yeast cell) is reduced or inhibited by providing within the cell a portion of the antisense strand of the *NMD2* gene introduced into cells in which *NMD2* is transcribed. The antisense oligonucleotide (either RNA or DNA) can be directly introduced into the cells in a form that is capable of binding to the *NMD2* sense transcripts. Alternatively, a vector containing sequence which, once within the host cells, is transcribed into the appropriate antisense mRNA, can be the species administered to the cells. An antisense nucleic acid which hybridizes to the mRNA of the target gene can

decrease or inhibit production of the polypeptide product encoded by the gene, by forming a double-stranded segment on the normally single-stranded mRNA transcript, and thereby interfering with translation.

A DNA sequence, such as a full or partial sequence of the *NMD2* gene, is expressed as an antisense transcript. The sequence can be operably linked to appropriate expression control sequences and introduced into host cells by standard techniques well known to those of ordinary skill in the art. An effective amount of the expressed antisense transcript is produced such that translation of the *NMD2* sense mRNA transcript is inhibited. By an equivalent method, *UPF1* expression is inhibited by the introduction of *UPF1* mRNA antisense transcript or a fragment thereof which binds to the *UPF1* sense transcript, inhibiting translation and thereby, inhibiting the nonsense-mediated mRNA pathway. Antisense transcript production can be constitutive or controlled, as desired, according to the transcription regulatory sequences operably linked to the *NMD2* or *UPF1* DNA sequences for the production of antisense transcript.

Inhibition of the nonsense-mediated mRNA pathway using antisense transcripts to inhibit translation of a protein of the pathway (such as *NMD2* or *UPF1*) is useful to enhance the stability of a nonsense codon-containing transcript which encodes a heterologous polypeptide to be produced in yeast cells or to enhance the production of a mutated endogenous polypeptide useful to the host cell or host organism.

Example 7: Production of Heterologous Protein or Polypeptide in a Yeast Cell Inhibited in the Nonsense-Mediated mRNA Pathway

A protein or polypeptide of interest is produced by providing an expression vector encoding a gene for a heterologous protein. The expressed transcript of the

gene encodes a nonsense codon in a transcript destabilizing 5′ portion of the transcript such that the transcript is at least 2 fold less stable in a wild-type strain than in a nonsense-mediated mRNA decay-inhibited host strain. Nonsense-mediated mRNA decay is inhibited by 1) mutating the *NMD2* gene such that no functional Nmd2p is produced; 2) overexpressing a C-terminal fragment of Nmd2p such that the fragment binds to Upf1p inhibiting its function; or 3) expressing sufficient *NMD2* or *UPF1* antisense transcript to hybridize to *NMD2* or *UPF1* sense transcript preventing its translation into functional Nmd2p or Upf1p, respectively. All of these methods can be carried out by standard procedures.

If it desired that an amino acid be substituted at the nonsense codon position, then the host strain used is also an amino acid substitution suppressor strain. The suppressor strain is chosen such that a specific amino acid (dictated by the type of suppressor mutation in the host strain) is substituted at the nonsense codon. The substituted amino acid can be an amino acid encoded by the natural codon at that site. The substituted amino acid can be different from the naturally encoded amino acid if it is desired to test the affect of that amino acid on the conformation or activity of the encoded protein.

If the heterologous protein to be expressed is toxic to the host cell, inhibition of the nonsense-mediated mRNA decay pathway can be controlled by the inducible expression of, for example, Nmd2p C-terminal fragment or *NMD2* antisense transcript. Controllable inhibition of the decay pathway allows transcript stabilization and translation at a point in the host yeast cell culture growth such that maximum production of the toxic protein occurs prior to the death of the host cells.

Following inhibition of the nonsense-mediated mRNA pathway and translation of the stabilized nonsense codon-containing transcript into the desired heterologous protein or protein fragment is isolated from the yeast host cells by standard protein purification methods.

Example 8: Production of Antibody to Nmd2p or a C-terminal Fragment of Nmd2p

Nmd2p or Nmd2p C-terminal fragment polypeptide of the invention can be produced by first transforming a suitable host cell with the entire NMD2 gene (for the production of Nmd2p) or with a partial NMD2 sequence (encoding the C-terminal part of Nmd2p), respectively, cloned into a suitable expression vehicle followed by expression of the desired protein or polypeptide.

Those of ordinary skill in the field of molecular biology will understand that any of a wide variety of expression systems can be used to provide the protein or polypeptide. The precise host cell used is not critical to the invention. The polypeptide can be produced in a prokaryotic host (e.g. *E. coli*) or in a eukaryotic host (e.g., *Saccharomyces cerevisiae*). The method of transformation of the cells and the choice of expression vehicle will depend on the host system selected. Methods described herein provide sufficient guidance to successfully carry out the production, purification and identification of Nmd2p or THE Nmd2p C-terminal fragment.

Once the Nmd2p or Nmd2p C-terminal fragment (or fragment or analog thereof) is expressed, it is isolated, e.g., using immunoaffinity chromatography. In one example, an anti-Nmd2p or anti-(Nmd2p C-terminal fragment) antibody can be attached to a column and used to isolate Nmd2p or Nmd2p C-terminal fragment, respectively. Lysis and fractionation of Nmd2p or Nmd2p C-terminal fragment-containing host cells prior to affinity chromatography can be performed by standard

methods. Once isolated, the recombinant protein can, if desired, be further purified, e.g., by high performance liquid chromatography (see, e.g., Fisher, Laboratory Techniques In Biochemistry and Molecular Biology, eds., Work and Burdon, Elsevier, (1980)).

Nmd2p or fragments thereof, particularly short fragments which inhibit the nonsense-mediated mRNA decay pathway, can also be produced by chemical synthesis by standard solution or solid phase peptide synthesis techniques.

Substantially pure Nmd2p or Nmd2p C-terminal fragment can be used to raise antibodies. The antibodies are useful for screening, by Western blot analysis, host strains overexpressing Nmd2p or Nmd2p C-terminal fragment, thereby identifying candidate strains which produce a desired amount of Nmd2p or Nmd2p C-terminal fragment.

Antibodies directed to the polypeptide of interest, Nmd2p or Nmd2p C-terminal fragment, are produced as follows. Peptides corresponding to all or part of the polypeptide of interest are produced using a peptide synthesizer by standard techniques, or are isolated and purified as described above. The peptides are coupled to KLH with m-maleimide benzoic acid N-hydroxysuccinimide ester. The KLH-peptide is mixed with Freund's adjuvant and injected into animals, e.g. guinea pigs or goats, to produce polyclonal antibodies.

Monoclonal antibodies can be prepared using the polypeptide of interest described above and standard hybridoma technology (see, e.g., Kohler et al., Nature (1975) 256:495; Kohler et al., Eur. J. Immunol. (1976) 6:292; Kohler et al., Eur. J. Immunol. (1976) 6:511; Hammerling et al., in Monoclonal Antibodies and T Cell Hybridomas, Elsevier, NY, (1981)). Antibodies are purified by peptide antigen affinity chromatography.

Once produced, antibodies are tested for specific Nmd2p or Nmd2p C-terminal fragment binding by Western blot or immunoprecipitation analysis by standard techniques.

USE

Overexpressing the C-terminal truncated form of Nmd2p in a cell (such as a yeast cell) provides for the inhibition of the nonsense-mediated mRNA decay pathway. Disruption or mutation of the *NMD2* gene or NMD2 antisense transcript expression are other methods for inhibiting the nonsense-mediated mRNA decay pathway. As a result, a transcript for a heterologous protein which contains at least one stop codon within a transcript-destabilizing 5′ portion will be specifically stabilized when expressed in a host cell inhibited in a nonsense-mediated mRNA decay pathway. Such stabilization allows translation of the stabilized transcript in a yeast suppressor mutant to produce a full length peptide with an amino acid inserted at the position of the nonsense codon. The inserted amino acid is specific to the suppressor mutant host in which the heterologous gene and the Nmd2p C-terminus are expressed. The relevant properties of each of the mutant heterologous proteins are compared to the properties of the wild-type protein, and altered heterologous proteins having desired properties are collected. Such properties may include but are not limited to protein receptor binding, antibody binding, enzymatic activity, three-dimensional structure, and other biological and physical properties known to those of ordinary skill in the arts of biochemistry and protein chemistry.

The invention is also useful in the production of heterologous protein fragments by inserting into the DNA a stop codon within a transcript-destabilizing 5′ portion of the coding sequence at a site at which translation is

to stop thereby producing an N-terminal protein fragment. Fragments useful in pharmaceutical or other applications can be isolated in large quantities if so desired by techniques well known to those of ordinary skill in the art.

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, that the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: UNIVERSITY OF MASSACHUSETTS MEDICAL SCHOOL

(ii) TITLE OF INVENTION: HETEROLOGOUS POLYPEPTIDE PRODUCTION IN THE ABSENCE OF NONSENSE-MEDIATED mRNA DECAY FUNCTION

(iii) NUMBER OF SEQUENCES: 6

(iv) CORRESPONDENCE ADDRESS:
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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
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(B) FILING DATE: 27-DEC-1995

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(A) APPLICATION NUMBER: US 08/375,300
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(viii) ATTORNEY/AGENT INFORMATION:
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(C) REFERENCE/DOCKET NUMBER: 04020/046WO1

(ix) TELECOMMUNICATION INFORMATION:
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(B) TELEFAX: (617)542-8906
(C) TELEX: 200154

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4080 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCTGAATGAC CTTTATCTTA ATTATGCACC ATCATATAGC GTTTCTATGA TCACTACGGG 60

ATTATTATGAT ATTGTTAGGG GGTTATATTG AATATTTCTT AGGGCATGAG GATGATATTA 120

GGGTTATTAA TAGGTTTACA ATTATATAAT TTATGTGATA ATTATCACTT GATACGAATT 180

GATGGAGCCT GCTTCTTTTT TTTTTTTTCA CTTTCTTGGC AGTCACTGAA AAACTGCATT 240

CGAATACAGG TTTGAGAAAC TAATGAGGCC CATATTACTT TACAATGAAC AGTAACAATC 300

AACTTAAATG CTTAAATAAT CTAATATTGT ATCTGCATTG ATAATACATT GGACAGAAAT 360

ATGGACGTA TGTTTGATTT ATCTTACTGT GGCCAGATCG GCCTTTCAGT ACTTCTAAGG 420

TTTTATACTA ACTTCTTTTA TTGATCGTTG TAAACTACGG TAACAATTAT GTATCAACAG 480

GATGGACGGA AAAAAGAATT GCATGATTTG AACACCCGAG CTTGGAATGG CGAAGAAGTC 540

TTTCCCCTGA AAAGTAAAAA ACTGGATTCC AGTATAAAGA GAAACACTGG CTTTATAAAA 600

AAACTAAAGA AGGGTTTTGT GAAAGGTTCA GAATCTTCAT TATTGAAAGA TTTAAGTGAG 660

CGTCCTTGG AAAAGTACCT ATCAGAGATA ATAGTGACGG TAACAGAATG TCTGCTAAAT 720

GTTTTGAATA AAAATGATGA CGTAATTGCC GCTGTTGAGA TCATAAGTGG ACTTCATCAA 780

AGGTTCAATG GCCGATTTAC TAGTCCGCTT TTAGGAGCTT TTTTACAAGC TTTTGAGAAC 840

CCCTCTGTTG ACATTGAATC CGAAAGAGAT GAGCTTCAAA GGATAACCAG AGTTAAAGGT 900

AATCTTCGGG TATTTACCGA GCTTTATTTA GTTGGAGTTT TTAGAACATT GGATGATATT 960

AGTCGAAAG ATGCTATTCC AAACTTCCTA CAGAAGAAAA CTGGGCGAAA GGATCCGTTG 1020

TTATTCAGTA TTCTCAGAGA GATTCTTAAT TATAAGTTCA AATTGGGCTT TACTACCACT 1080

ATTGCGACCG CATTTATTAA GAAATTTGCA CCTTTGTTTC GCGACGATGA TAATTCTTGG 1140

GATGATTTAA TATATGACTC GAAGTTAAAA GGTGCGTTAC AGTCTCTGTT TAAGAATTTT 1200

ATAGACGCCA CTTTTGCGAG GGCCACAGAA CTGCATAAGA AGGTCAATAA ACTGCAAAGA 1260

AACATCAGA AATGCCAAAT AAGAACGGGA AAATTGAGAG ATGAGTACGT AGAGGAGTAC 1320

GACAAGTTAC TTCCAATATT CATTAGGTTC AAGACATCTG CAATTACTTT GGGAGAATTT 1380

TTTAAGTTAG AAATTCCGGA GCTTGAAGGT GCCTCTAATG ATGATCTGAA AGAAACAGCT 1440

TCTCCAATGA TCACGAATCA GATATTGCCA CCCAACCAAC GATTATGGGA AAATGAAGAT 1500

ACAAGGAAAT TTTATGAAAT CTTACCAGAT ATCTCAAAAA CAGTAGAAGA ATCACAATCT 1560

CTAAAACAG AAAAAGATTC AAACGTTAAC TCAAAAAATA TCAATCTATT CTTTACGGAT 1620

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TATTTGGACA ACAAAGCCAC AAGAAATCGA ATATTGAAAT TTTTCATGGA AACACAAGAT 1740

TGGAGCAAAC TGCCAGTGTA TTCCAGATTT ATTGCAACAA ATAGCAAATA TATGCCGGAA 1800

ATTGTTTCTG AGTTTATTAA CTACCTAGAC AATGGCTTCA GGAGTCAATT ACATTCTAAT 1860

AAGATTAACG TTAAAAACAT CATCTTCTTC AGTGAAATGA TTAAATTTCA ATTAATACCA 1920

TCGTTTATGA TTTTTCATAA GATTAGAACA TTAATCATGT ATATGCAAGT TCCAAATAAC 1980

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GATAATTATT TCAGGATTCA ACTAGTCACT ACAATTCTGT TAAATATCAA CAGGACCCCT 2700

GCAGCTTTTA CTAAGAAATG CAAACTTTTG CTGAGGTTTT TCGAGTATTA TACTTTTATT 2760

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GACGATGATG ATGATGATGG AGAAGAAGGC GATGAGGATG ATGATGAAGA TGATGATGAT 3240

GAGGATGATG ATGATGAAGA AGAAGAAGAC AGCGACTCTG ATTTGGAGTA TGGTGGTGAT 3300

CTTGACGCAG ACAGAGATAT TGAAATGAAA CGAATGTATG AAGAGTACGA GAGAAAACTA 3360

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AGCAAGGAGA CCTACGAAGA GTTATCCAAG CCAAAGAAGA TTGCATTTAC GTTCTTGACT 3600

AAAAGCGGTA AGAAGACACA ATCAAGAATT TTACAATTAC CAACGGATGT GAAATTTGTC 3660

TCTGATGTCC TTGAAGAAGA AGAGAAACTA AAAACCGAGC GAAACAAGAT TAAAAAGATT 3720

GTTTTAAAAC GTTCTTTCGA CTGAGATTCT TTGCGAATAT AGTTCTTTAA ATTTTTACTA 3780

TATATGCCCA CTTATGTTTG GCTCTATTAA ATGGCTACGT GTTTATATAG TACCGTTTAT 3840

GACGCTGTAT TTTTATTTAC ACTGCTTTCC AGGAGATTAA AGAGCGGAGT GTTAGTCAAC 3900

TCTCACGACA ACAACAGTTA TATCGTCTTC TTTACCACCG CTGTAGTTTT TGCCAGTTAG 3960

CTTAGAAATC TCTTGCGCAA AAACACTGGG GTAATTGGGG TCCTTGCTTA AACTGACAAC 4020

ATTGTCCACA AACTTCTGGG ATAATAGCTG TAACTCATCG TTTGTTCTCG CAGCGTTATC 4080

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1089 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Asp Asp Gly Arg Lys Lys Glu Leu His Asp Leu Asn Thr Arg Ala
1 5 10 15

Trp Asn Gly Glu Glu Val Phe Pro Leu Lys Ser Lys Lys Leu Asp Ser
20 25 30

Ser Ile Lys Arg Asn Thr Gly Phe Ile Lys Lys Leu Lys Lys Gly Phe
35 40 45

Val Lys Gly Ser Glu Ser Ser Leu Leu Lys Asp Leu Ser Glu Ala Ser
50 55 60

Leu Glu Lys Tyr Leu Ser Glu Ile Ile Val Thr Val Thr Glu Cys Leu
65 70 75 80

Leu Asn Val Leu Asn Lys Asn Asp Asp Val Ile Ala Ala Val Glu Ile
85 90 95

Ile Ser Gly Leu His Gln Arg Phe Asn Gly Arg Phe Thr Ser Pro Leu
100 105 110

Leu Gly Ala Phe Leu Gln Ala Phe Glu Asn Pro Ser Val Asp Ile Glu
115 120 125

Ser Glu Arg Asp Glu Leu Gln Arg Ile Thr Arg Val Lys Gly Asn Leu
130 135 140

Arg Val Phe Thr Glu Leu Tyr Leu Val Gly Val Phe Arg Thr Leu Asp
145 150 155 160

Asp Ile Glu Ser Lys Asp Ala Ile Pro Asn Phe Leu Gln Lys Lys Thr
165 170 175

Gly Arg Lys Asp Pro Leu Leu Phe Ser Ile Leu Arg Glu Ile Leu Asn
180 185 190

Tyr Lys Phe Lys Leu Gly Phe Thr Thr Thr Ile Ala Thr Ala Phe Ile
195 200 205

Lys Lys Phe Ala Pro Leu Phe Arg Asp Asp Asp Asn Ser Trp Asp Asp
210 215 220

Leu Ile Tyr Asp Ser Lys Leu Lys Gly Ala Leu Gln Ser Leu Phe Lys
225 230 235 240

Asn Phe Ile Asp Ala Thr Phe Ala Arg Ala Thr Glu Leu His Lys Lys
245 250 255

Val Asn Lys Leu Gln Arg Glu His Gln Lys Cys Gln Ile Arg Thr Gly
260 265 270

Lys Leu Arg Asp Glu Tyr Val Glu Glu Tyr Asp Lys Leu Leu Pro Ile
275 280 285

Phe Ile Arg Phe Lys Thr Ser Ala Ile Thr Leu Gly Glu Phe Phe Lys
290 295 300

Leu Glu Ile Pro Glu Leu Glu Gly Ala Ser Asn Asp Asp Leu Lys Glu
305 310 315 320

Thr Ala Ser Pro Met Ile Thr Asn Gln Ile Leu Pro Pro Asn Gln Arg
325 330 335

Leu Trp Glu Asn Glu Asp Thr Arg Lys Phe Tyr Glu Ile Leu Pro Asp
340 345 350

Ile Ser Lys Thr Val Glu Glu Ser Gln Ser Ser Lys Thr Glu Lys Asp
355 360 365

Ser Asn Val Asn Ser Lys Asn Ile Asn Leu Phe Phe Thr Asp Leu Glu
370 375 380

Met Ala Asp Cys Lys Asp Ile Ile Asp Asp Leu Ser Asn Arg Tyr Trp
385 390 395 400

Ser Ser Tyr Leu Asp Asn Lys Ala Thr Arg Asn Arg Ile Leu Lys Phe
405 410 415

Phe Met Glu Thr Gln Asp Trp Ser Lys Leu Pro Val Tyr Ser Arg Phe
420 425 430

Ile Ala Thr Asn Ser Lys Tyr Met Pro Glu Ile Val Ser Glu Phe Ile
435 440 445

Asn Tyr Leu Asp Asn Gly Phe Arg Ser Gln Leu His Ser Asn Lys Ile
450 455 460

Asn Val Lys Asn Ile Ile Phe Phe Ser Glu Met Ile Lys Phe Gln Leu
465 470 475 480

Ile Pro Ser Phe Met Ile Phe His Lys Ile Arg Thr Leu Ile Met Tyr
485 490 495

Met Gln Val Pro Asn Asn Val Glu Ile Leu Thr Val Leu Leu Glu His
500 505 510

Ser Gly Lys Phe Leu Leu Asn Lys Pro Glu Tyr Lys Glu Leu Met Glu
515 520 525

Lys Met Val Gln Leu Ile Lys Asp Lys Lys Asn Asp Arg Gln Leu Asn
530 535 540

Met Asn Met Lys Ser Ala Leu Glu Asn Ile Ile Thr Leu Leu Tyr Pro
545 550 555 560

Pro Ser Val Lys Ser Leu Asn Val Thr Val Lys Thr Ile Thr Pro Glu
565 570 575

Gln Gln Phe Tyr Arg Ile Leu Ile Arg Ser Glu Leu Ser Ser Leu Asp
580 585 590

Phe Lys His Ile Val Lys Leu Val Arg Lys Ala His Trp Asp Asp Val
595 600 605

Ala Ile Gln Lys Val Leu Phe Ser Leu Phe Ser Lys Pro His Lys Ile
610 615 620

Ser Tyr Gln Asn Ile Pro Leu Leu Thr Lys Val Leu Gly Gly Leu Tyr
625 630 635 640

Ser Tyr Arg Arg Asp Phe Val Ile Arg Cys Ile Asp Gln Val Leu Glu
645 650 655

Asn Ile Glu Arg Gly Leu Glu Ile Asn Asp Tyr Gly Gln Asn Met His
660 665 670

Arg Ile Ser Asn Val Arg Tyr Leu Thr Glu Ile Phe Asn Phe Glu Met
675 680 685

Ile Lys Ser Asp Val Leu Leu Asp Thr Ile Tyr His Ile Ile Arg Phe
690 695 700

Gly His Ile Asn Asn Gln Pro Asn Pro Phe Tyr Leu Asn Tyr Ser Asp
705 710 715 720

Pro Pro Asp Asn Tyr Phe Arg Ile Gln Leu Val Thr Thr Ile Leu Leu
725 730 735

Asn Ile Asn Arg Thr Pro Ala Ala Phe Thr Lys Lys Cys Lys Leu Leu
740 745 750

Leu Arg Phe Phe Glu Tyr Tyr Thr Phe Ile Lys Glu Gln Pro Leu Pro
755 760 765

Lys Glu Thr Glu Phe Arg Val Ser Ser Thr Phe Lys Lys Tyr Glu Asn
770 775 780

Ile Phe Gly Asn Thr Lys Phe Glu Arg Ser Glu Asn Leu Val Glu Ser
785 790 795 800

Ala Ser Arg Leu Glu Ser Leu Leu Lys Ser Leu Asn Ala Ile Lys Ser
805 810 815

Lys Asp Asp Arg Val Lys Gly Ser Ser Ala Ser Ile His Asn Gly Lys
820 825 830

Glu Ser Ala Val Pro Ile Glu Ser Ile Thr Glu Asp Asp Glu Asp Glu
835 840 845

Asp Asp Glu Asn Asp Asp Gly Val Asp Leu Leu Gly Glu Asp Glu Asp
850 855 860

Ala Glu Ile Ser Thr Pro Asn Thr Glu Ser Ala Pro Gly Lys His Gln
865 870 875 880

Ala Lys Gln Asp Glu Ser Glu Asp Glu Asp Asp Glu Asp Asp Asp Glu
885 890 895

Asp Asp Asp Asp Asp Asp Asp Asp Asp Asp Asp Asp Gly Glu Glu Gly
900 905 910

Asp Glu Asp Asp Asp Glu Asp Asp Asp Asp Glu Asp Asp Asp Asp Glu
915 920 925

Glu Glu Glu Asp Ser Asp Ser Asp Leu Glu Tyr Gly Gly Asp Leu Asp
930 935 940

Ala Asp Arg Asp Ile Glu Met Lys Arg Met Tyr Glu Glu Tyr Glu Arg
945 950 955 960

Lys Leu Lys Asp Glu Glu Glu Arg Lys Ala Glu Glu Glu Leu Glu Arg
965 970 975

Gln Phe Gln Lys Met Met Gln Glu Ser Ile Asp Ala Arg Lys Ser Glu
980 985 990

Lys Val Val Ala Ser Lys Ile Pro Val Ile Ser Lys Pro Val Ser Val
995 1000 1005

Gln Lys Pro Leu Leu Leu Lys Lys Ser Glu Glu Pro Ser Ser Ser Lys
1010 1015 1020

Glu Thr Tyr Glu Glu Leu Ser Lys Pro Lys Lys Ile Ala Phe Thr Phe
1025 1030 1035 1040

Leu Thr Lys Ser Gly Lys Lys Thr Gln Ser Arg Ile Leu Gln Leu Pro
1045 1050 1055

Thr Asp Val Lys Phe Val Ser Asp Val Leu Glu Glu Glu Glu Lys Leu
1060 1065 1070

Lys Thr Glu Arg Asn Lys Ile Lys Lys Ile Val Leu Lys Arg Ser Phe
1075 1080 1085

Asp

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2295 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATCACGAATC AGATATTGCC ACCCAACCAA CGATTATGGG AAAATGAAGA TACAAGGAAA 60

TTTTATGAAA TCTTACCAGA TATCTCAAAA ACAGTAGAAG AATCACAATC TTCTAAAACA 120

GAAAAAGATT CAAACGTTAA CTCAAAAAAT ATCAATCTAT TCTTTACGGA TTTGGAAATG 180

GCAGATTGTA AAGATATAAT CGATGACCTT TCAAATAGAT ATTGGTCATC ATATTTGGAC 240

AACAAAGCCA CAAGAAATCG AATATTGAAA TTTTTCATGG AAACACAAGA TTGGAGCAAA 300

CTGCCAGTGT ATTCCAGATT TATTGCAACA AATAGCAAAT ATATGCCGGA AATTGTTTCT 360

GAGTTTATTA ACTACCTAGA CAATGGCTTC AGGAGTCAAT TACATTCTAA TAAGATTAAC 420

GTTAAAAACA TCATCTTCTT CAGTGAAATG ATTAAATTTC AATTAATACC ATCGTTTATG 480

ATTTTTCATA AGATTAGAAC ATTAATCATG TATATGCAAG TTCCAAATAA CGTAGAAATT 540

TTGACGGTTT TGTTGGAGCA CTCAGGGAAA TTTCTGCTAA ATAAGCCAGA ATATAAGGAA 600

TTAATGGAAA AAATGGTCCA ACTAATCAAG GATAAAAAAA ATGATAGGCA ATTGAACATG 660

AACATGAAAA GCGCCTTAGA AAACATAATT ACTTTACTTT ATCCCCCTTC TGTAAAATCA 720

TTAAATGTTA CGGTAAAAAC AATAACGCCT GAACAACAGT TTTATCGCAT ATTAATTAGA 780

AGTGAACTAA GTAGCCTAGA CTTCAAACAC ATTGTCAAGT TGGTTCGGAA AGCTCACTGG 840

GACGATGTAG CTATTCAGAA AGTGCTGTTT TCTCTGTTTT CAAAACCACA TAAGATTAGC 900

TATCAAAATA TTCCCTTATT AACAAAAGTT CTAGGCGGTC TATACAGTTA CCGCCGCGAT 960

ATCGTCATCA GATGTATAGA CCAAGTACTG GAAAACATTG AGCGAGGCTT AGAAATTAAC 1020

GATTATGGAC AAAACATGCA TAGAATATCA AATGTCAGAT ACTTAACTGA AATATTCAAC 1080

TTTGAAATGA TAAAATCCGA TGTTTTGTTA GATACTATCT ACCACATTAT TCGGTTTGGT 1140

CATATCAACA ATCAACCCAA TCCATTTTAT TTAAACTACT CAGATCCACC GGATAATTAT 1200

TTCAGGATTC AACTAGTCAC TACAATTCTG TTAAATATCA ACAGGACCCC TGCAGCTTTT 1260

ACCTAAGAAAT GCAAACTTTT GCTGAGGTTT TTCGAGTATT ATACTTTTAT TAAAGAACAA 1320

CCTTTACCCA AGGAAACAGA ATTCAGAGTT TCAAGCACAT TTAAAAAATA TGAGAATATT 1380

TTCGGAAACA CTAAATTTGA AAGGTCAGAA AATTTGGTAG AAAGTGCCTC AAGGTTGGAA 1440

AGTTTACTGA AATCATTAAA CGCAATAAAA AGTAAAGACG ACAGAGTGAA GGGATCTTCT 1500

GCAAGCATTC ACAACGGTAA GGAGAGTGCT GTTCCTATCG AGTCAATCAC CGAAGATGAT 1560

AGGATGAAG ATGATGAAAA CGACGATGGT GTCGATTTAC TAGGAGAAGA TGAAGACGCG 1620

GAGATAAGTA CACCGAACAC AGAGTCAGCG CCAGGAAAAC ATCAGGCAAA GCAAGACGAA 1680

AGTGAAGATG AAGACGATGA GGACGATGAC GAGGATGATG ACGATGACGA TGACGATGAT 1740

GATGATGATG GAGAAGAAGG CGATGAGGAT GATGATGAAG ATGATGATGA TGAGGATGAT 1800

GATGATGAAG AAGAAGAAGA CAGCGACTCT GATTTGGAGT ATGGTGGTGA TCTTGACGCA 1860

ACACAGAGATA TTGAAATGAA ACGAATGTAT GAAGAGTACG AGAGAAAACT AAAGGATGAG 1920

GAAGAAAGGA AAGCGGAAGA AGAATTGGAA AGGCAATTTC AGAAAATGAT GCAAGAATCC 1980

ATAGACGCAA GGAAAAGCGA AAAGGTTGTT GCCAGTAAAA TTCCAGTAAT TTCGAAGCCA 2040

GTCAGCGTTC AAAAACCTTT ATTATTAAAA AAGAGTGAAG AACCTTCTTC AAGCAAGGAG 2100

ACCTACGAAG AGTTATCCAA GCCAAAGAAG ATTGCATTTA CGTTCTTGAC TAAAAGCGGT 2160

AAGAAGACAC AATCAAGAAT TTTACAATTA CCAACGGATG TGAAATTTGT CTCTGATGTC 2220

CTTGAAGAAG AAGAGAAACT AAAAACCGAG CGAAACAAGA TTAAAAAGAT TGTTTTAAAA 2280

CGTTCTTTCG ACTGA 2295

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 764 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Ile Thr Asn Gln Ile Leu Pro Pro Asn Gln Arg Leu Trp Glu Asn Glu
1 5 10 15

Asp Thr Arg Lys Phe Tyr Glu Ile Leu Pro Asp Ile Ser Lys Thr Val
20 25 30

Glu Glu Ser Gln Ser Ser Lys Thr Glu Lys Asp Ser Asn Val Asn Ser
35 40 45

Lys Asn Ile Asn Leu Phe Phe Thr Asp Leu Glu Met Ala Asp Cys Lys
50 55 60

Asp Ile Ile Asp Asp Leu Ser Asn Arg Tyr Trp Ser Ser Tyr Leu Asp
65 70 75 80

Asn Lys Ala Thr Arg Asn Arg Ile Leu Lys Phe Phe Met Glu Thr Gln
85 90 95

Asp Trp Ser Lys Leu Pro Val Tyr Ser Arg Phe Ile Ala Thr Asn Ser
100 105 110

Lys Tyr Met Pro Glu Ile Val Ser Glu Phe Ile Asn Tyr Leu Asp Asn
115 120 125

Gly Phe Arg Ser Gln Leu His Ser Asn Lys Ile Asn Val Lys Asn Ile
130 135 140

Ile Phe Phe Ser Glu Met Ile Lys Phe Gln Leu Ile Pro Ser Phe Met
145 150 155 160

Ile Phe His Lys Ile Arg Thr Leu Ile Met Tyr Met Gln Val Pro Asn
165 170 175

Asn Val Glu Ile Leu Thr Val Leu Leu Glu His Ser Gly Lys Phe Leu
180 185 190

Leu Asn Lys Pro Glu Tyr Lys Glu Leu Met Glu Lys Met Val Gln Leu
195 200 205

Ile Lys Asp Lys Lys Asn Asp Arg Gln Leu Asn Met Asn Met Lys Ser
210 215 220

Ala Leu Glu Asn Ile Ile Thr Leu Leu Tyr Pro Pro Ser Val Lys Ser
225 230 235 240

Leu Asn Val Thr Val Lys Thr Ile Thr Pro Glu Gln Gln Phe Tyr Arg
245 250 255

Ile Leu Ile Arg Ser Glu Leu Ser Ser Leu Asp Phe Lys His Ile Val
260 265 270

Lys Leu Val Arg Lys Ala His Trp Asp Asp Val Ala Ile Gln Lys Val
275 280 285

Leu Phe Ser Leu Phe Ser Lys Pro His Lys Ile Ser Tyr Gln Asn Ile
290 295 300

Pro Leu Leu Thr Lys Val Leu Gly Gly Leu Tyr Ser Tyr Arg Arg Asp
305 310 315 320

Phe Val Ile Arg Cys Ile Asp Gln Val Leu Glu Asn Ile Glu Arg Gly
325 330 335

Leu Glu Ile Asn Asp Tyr Gly Gln Asn Met His Arg Ile Ser Asn Val
340 345 350

Arg Tyr Leu Thr Glu Ile Phe Asn Phe Glu Met Ile Lys Ser Asp Val
355 360 365

Leu Leu Asp Thr Ile Tyr His Ile Ile Arg Phe Gly His Ile Asn Asn
370 375 380

Gln Pro Asn Pro Phe Tyr Leu Asn Tyr Ser Asp Pro Pro Asp Asn Tyr
385 390 395 400

Phe Arg Ile Gln Leu Val Thr Thr Ile Leu Leu Asn Ile Asn Arg Thr
405 410 415

Pro Ala Ala Phe Thr Lys Lys Cys Lys Leu Leu Leu Arg Phe Phe Glu
420 425 430

Tyr Tyr Thr Phe Ile Lys Glu Gln Pro Leu Pro Lys Glu Thr Glu Phe
435 440 445

Arg Val Ser Ser Thr Phe Lys Lys Tyr Glu Asn Ile Phe Gly Asn Thr
450 455 460

Lys Phe Glu Arg Ser Glu Asn Leu Val Glu Ser Ala Ser Arg Leu Glu
465 470 475 480

Ser Leu Leu Lys Ser Leu Asn Ala Ile Lys Ser Lys Asp Asp Arg Val
485 490 495

Lys Gly Ser Ser Ala Ser Ile His Asn Gly Lys Glu Ser Ala Val Pro
500 505 510

Ile Glu Ser Ile Thr Glu Asp Asp Glu Asp Glu Asp Asp Glu Asn Asp
515 520 525

Asp Gly Val Asp Leu Leu Gly Glu Asp Glu Asp Ala Glu Ile Ser Thr
530 535 540

Pro Asn Thr Glu Ser Ala Pro Gly Lys His Gln Ala Lys Gln Asp Glu
545 550 555 560

Ser Glu Asp Glu Asp Asp Glu Asp Asp Asp Glu Asp Asp Asp Asp Asp
565 570 575

Asp Asp Asp Asp Asp Asp Asp Gly Glu Glu Gly Asp Glu Asp Asp Asp
580 585 590

Glu Asp Asp Asp Asp Glu Asp Asp Asp Asp Glu Glu Glu Glu Asp Ser
595 600 605

Asp Ser Asp Leu Glu Tyr Gly Gly Asp Leu Asp Ala Asp Arg Asp Ile
610 615 620

Glu Met Lys Arg Met Tyr Glu Glu Tyr Glu Arg Lys Leu Lys Asp Glu
625 630 635 640

Glu Glu Arg Lys Ala Glu Glu Glu Leu Glu Arg Gln Phe Gln Lys Met
645 650 655

Met Gln Glu Ser Ile Asp Ala Arg Lys Ser Glu Lys Val Val Ala Ser
660 665 670

Lys Ile Pro Val Ile Ser Lys Pro Val Ser Val Gln Lys Pro Leu Leu
675 680 685

Leu Lys Lys Ser Glu Glu Pro Ser Ser Ser Lys Glu Thr Tyr Glu Glu
690 695 700

Leu Ser Lys Pro Lys Lys Ile Ala Phe Thr Phe Leu Thr Lys Ser Gly
705 710 715 720

Lys Lys Thr Gln Ser Arg Ile Leu Gln Leu Pro Thr Asp Val Lys Phe
725 730 735

Val Ser Asp Val Leu Glu Glu Glu Glu Lys Leu Lys Thr Glu Arg Asn
740 745 750

Lys Ile Lys Lys Ile Val Leu Lys Arg Ser Phe Asp
755 760

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCGGAATTCA TGGTCGGTTC CGGTTCT 27

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AGTGACTTGA GCCTC 15

What is claimed is:

Claims

1. Substantially pure DNA comprising the sequence of SEQ ID NO:1, or degenerate variants thereof.

2. Substantially pure DNA comprising the 3' terminus of the DNA sequence, SEQ ID NO:3, or degenerate variants thereof.

3. A vector comprising the DNA of any one of claims 1 or 2 operably linked to transcriptional regulatory sequences for expression of sense transcript.

4. A vector comprising the DNA of any one of claims 1 or 2 operably linked to transcriptional regulatory sequences for expression of antisense transcript.

5. A cell which contains the DNA of any one of claims 1 or 2.

6. A cell which contains the vector of any one of claims 3 or 4.

7. Substantially pure nonsense-mediated mRNA decay pathway protein, Nmd2p, from the genus, *Saccharomyces*.

8. A substantially pure polypeptide comprising the amino acid sequence of SEQ ID NO:2.

9. A substantially pure polypeptide comprising a C-terminal fragment of Nmd2p, wherein:

a) said polypeptide binds to Upf1p; and

b) said polypeptide substantially inhibits nonsense-mediated mRNA decay when said polypeptide is overexpressed in said cell.

10. A substantially pure polypeptide comprising the amino acid sequence of SEQ ID NO:4.

11. A method of substantially inhibiting nonsense-mediated mRNA decay in a cell, said method comprising:

a) providing a cell containing the DNA of claim 2;

b) overexpressing said DNA in said cell to produce an overexpressed polypeptide that binds to Upf1p and interferes with Upf1p function.

12. A method of substantially inhibiting nonsense-mediated mRNA decay in a cell, said method comprising:

a) providing a cell containing an *NMD2* gene;

b) mutating said *NMD2* gene such that essentially no functional Nmd2p is produced in said cell.

13. A method of substantially inhibiting nonsense-mediated mRNA decay in a cell, said method comprising:

a) providing a cell of claim 6;

b) expressing antisense transcript of *NMD2* in sufficient amount to bind to the *NMD2* transcript.

14. A method of producing a heterologous polypeptide from an mRNA transcript, said transcript containing at least one nonsense codon in a transcript destabilizing 5′ portion, said method comprising:

a) providing a cell containing DNA of claim 2;

b) overexpressing said DNA in said cell to produce a first polypeptide that substantially inhibits nonsense-mediated mRNA decay in said cell;

c) expressing a gene encoding said heterologous polypeptide in said cell to produce said mRNA transcript wherein said transcript stability is substantially increased; and

d) translating said transcript in said cell to produce said heterologous polypeptide.

15. A method of producing a heterologous polypeptide from an mRNA transcript, said transcript containing at least one nonsense codon in a transcript destabilizing 5′ portion, said method comprising:

a) providing a cell containing the *NMD2* gene;

b) mutating the *NMD2* gene in said cell such that no functional Nmd2p is produced and nonsense-mediated mRNA decay in said cell is substantially inhibited;

c) expressing a gene encoding said heterologous polypeptide in said cell to produce said mRNA transcript wherein said transcript stability is substantially increased; and

d) translating said transcript in said cell to produce said heterologous polypeptide.

16. A method of producing a heterologous polypeptide from an mRNA transcript, said transcript containing at least one nonsense codon in a transcript destabilizing 5′ portion, said method comprising:

a) providing a cell containing the *UPF1* gene;

b) mutating the *UPF1* gene in said cell such that no functional Upf1p is produced and nonsense-mediated mRNA decay in said cell is substantially inhibited;

c) expressing a gene encoding said heterologous polypeptide in said cell to produce said mRNA transcript

wherein said transcript stability is substantially increased; and

d) translating said transcript in said cell to produce said heterologous polypeptide.

17. A method of producing a heterologous polypeptide from an mRNA transcript, said transcript containing at least one nonsense codon in a transcript destabilizing 5′ portion, said method comprising:

a) providing a cell of claim 6;

b) expressing an antisense transcript of *NMD2* in said cell such that no functional Nmd2p is produced and nonsense-mediated mRNA decay in said cell is substantially inhibited;

c) expressing a gene encoding said heterologous polypeptide in said cell to produce said mRNA transcript wherein said transcript stability is substantially increased; and

d) translating said transcript in said cell to produce said heterologous polypeptide.

18. A method of producing a heterologous polypeptide from an mRNA transcript, said transcript containing at least one nonsense codon in a transcript destabilizing 5′ portion, said method comprising:

a) providing a cell containing a vector encoding *UPF1* operably linked to transcriptional regulatory sequences for controlled expression of antisense transcript;

b) expressing said antisense transcript of *UPF1* in said cell such that no functional Upf1p is produced and nonsense-mediated mRNA decay in said cell is substantially inhibited;

c) expressing a gene encoding said heterologous polypeptide in said cell to produce said mRNA transcript

wherein said transcript stability is substantially increased; and

d) translating said transcript in said cell to produce said heterologous polypeptide.

19. A method of claim 18, wherein said cell is a nonsense suppressor mutant which inserts a known amino acid into the position of a nonsense codon.

20. A method of claim 18, wherein said cell does not suppress said nonsense codon and said heterologous polypeptide is an N-terminal fragment of a full length protein.

21. A substantially pure polypeptide that binds to the Upf1p protein wherein the binding causes inhibition of the nonsense mediated mRNA decay pathway.

22. An antibody which specifically binds to the protein of any one of claims 7, 8, or 9.

23. A method of screening a candidate host cell for the amount of Nmd2p produced by said cell relative to a control cell, said method comprising:

a) providing a clonal population of said candidate host cell;

b) treating said clonal population of cells such that the intracellular proteins are accessible to an antibody;

c) contacting said intracellular proteins with an antibody that specifically binds to Nmd2p protein of claim 7; and

d) determining the relative amount of Nmd2p produced by said candidate host cell.

24. A method of screening a candidate host cell for the amount of polypeptide comprising the amino acid sequence of SEQ ID NO:2, produced by said cell relative to a control cell, said method comprising:

a) providing a clonal population of said candidate host cell;

b) treating said clonal population of cells such that the intracellular proteins are accessible to an antibody;

c) contacting said intracellular proteins with an antibody that specifically binds to a polypeptide of claim 8; and

d) determining the relative amount of polypeptide comprising the of amino acid sequence of SEQ ID NO:2, produced by said candidate host cell.

25. A method of screening a candidate host cell for the amount of polypeptide comprising the amino acid sequence of SEQ ID NO:4 produced by said cell relative to a control cell, said method comprising:

a) providing a clonal population of said candidate host cell;

b) treating said clonal population of cells such that the intracellular proteins are accessible to an antibody;

c) contacting said intracellular proteins with an antibody that specifically binds to a polypeptide of claim 9; and

d) determining the relative amount of polypeptide comprising the of amino acid sequence of SEQ ID NO:4, produced by said candidate host cell.

HETEROLOGOUS POLYPEPTIDE PRODUCTION IN THE ABSENCE OF NONSENSE-MEDIATED mRNA DECAY FUNCTION

Abstract of the Disclosure

The invention relates to the discovery of a gene, *NMD2*, named after its role in the Nonsense-Mediated mRNA Decay pathway, and the protein, Nmd2p, encoded by the *NMD2* gene. The amino acid sequence of Nmd2p and the nucleotide sequence of the *NMD2* gene encoding it are disclosed. Nmd2p is shown herein to bind to another protein in the decay pathway, Upf1p. A C-terminal fragment of the protein is also shown to bind Upf1p and, when overexpressed in the host cell, the fragment inhibits the function of Upf1p, thereby inhibiting the nonsense-mediated mRNA decay pathway. The invention also relates to methods of inhibiting the nonsense-mediated mRNA decay pathway to stabilize mRNA transcripts containing a nonsense codon which normally would cause an increase in the transcript decay rate. Such stabilization of a transcript is useful for the production of a recombinant protein or fragment thereof.

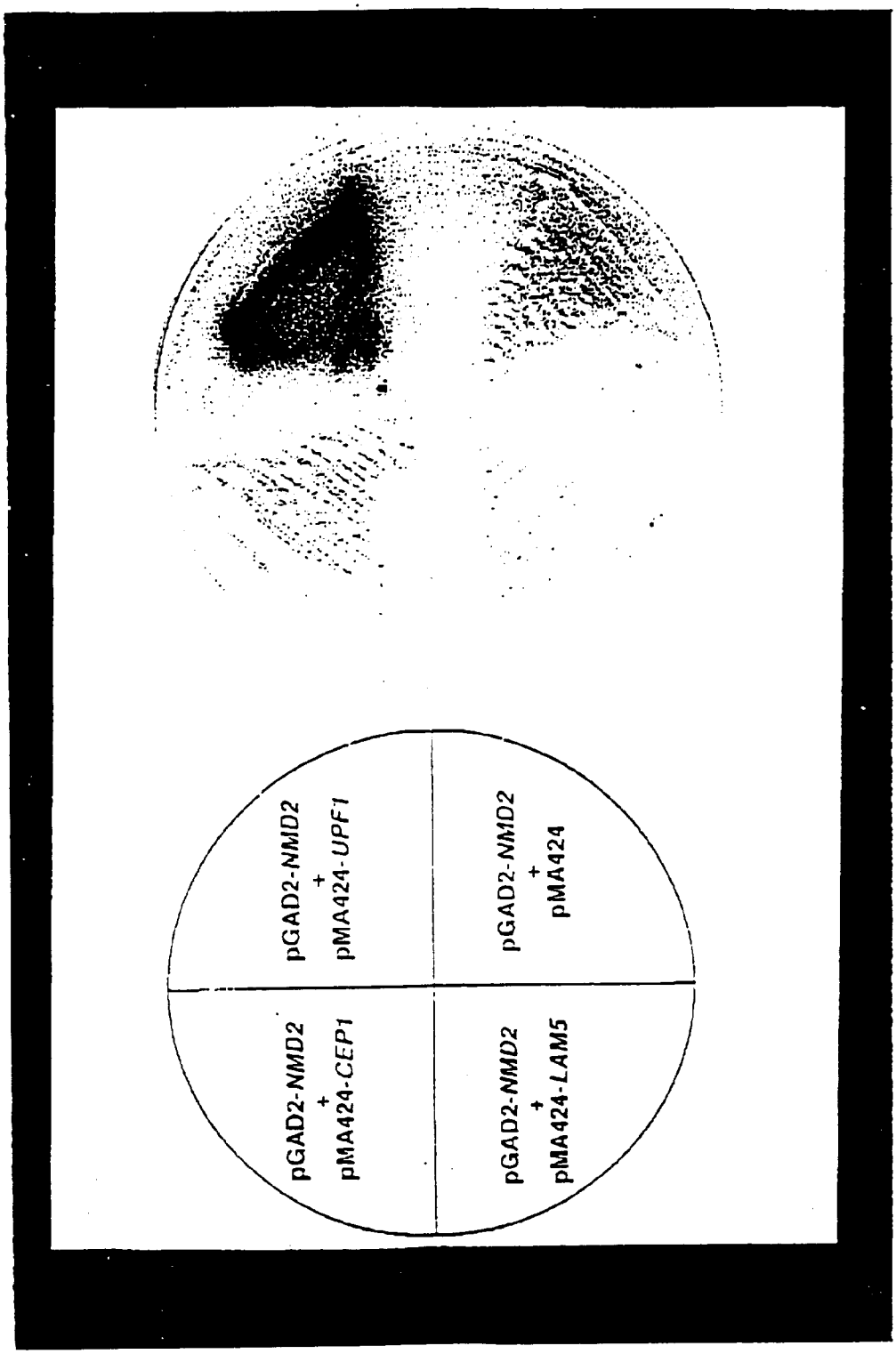
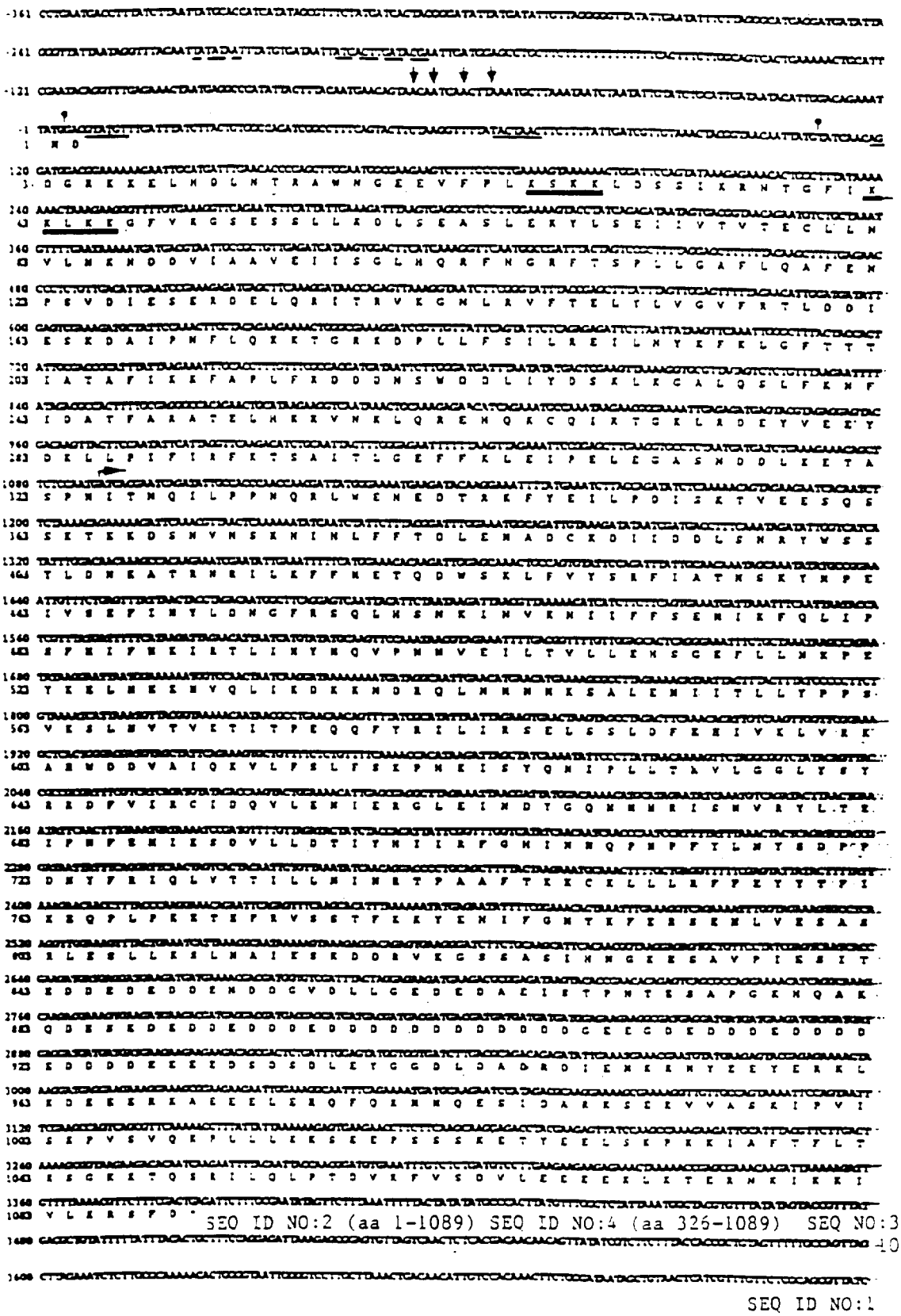

pGAD2-NMD2 + pMA424-UPF1
pGAD2-NMD2 + pMA424
pGAD2-NMD2 + pMA424-CEP1
pGAD2-NMD2 + pMA424-LAM5

FIGURE 1

FIGURE 2



SEQ ID NO:2 (aa 1-1089) SEQ ID NO:4 (aa 326-1089) SEQ NO:3 (nt 1089-338

SEQ ID NO:1

FIGURE 3A

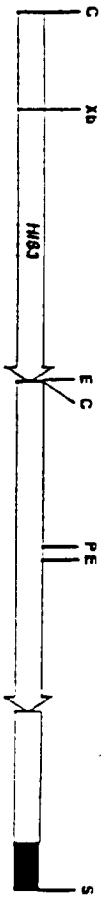


FIGURE 3B

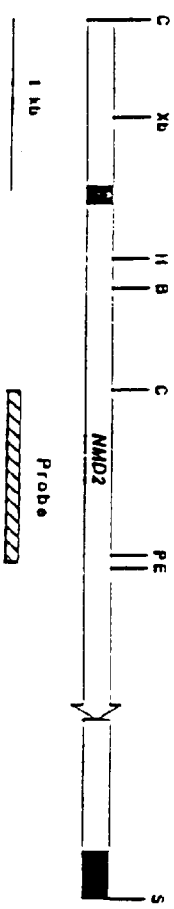
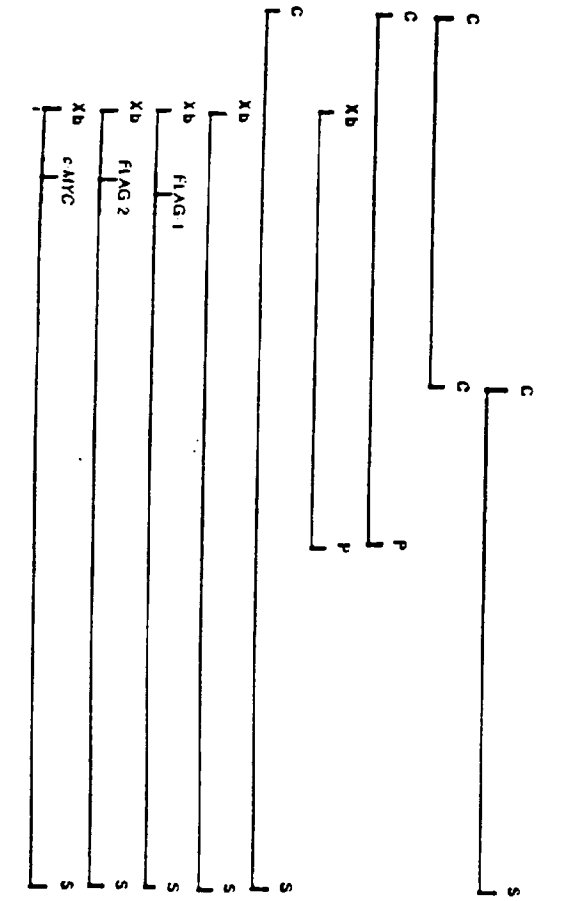


FIGURE 3C



Complementation

+ + + + + − − − −

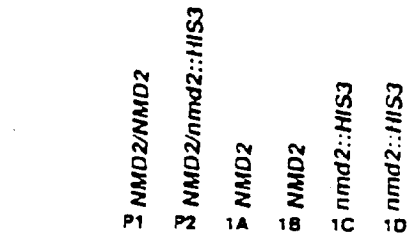


FIGURE 4A

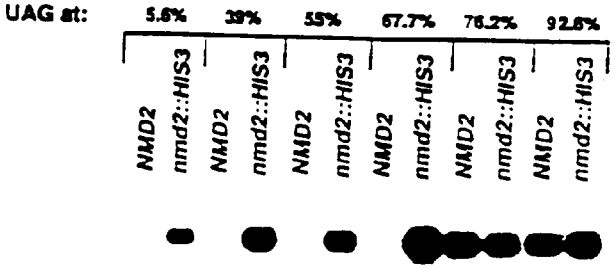


FIGURE 4B

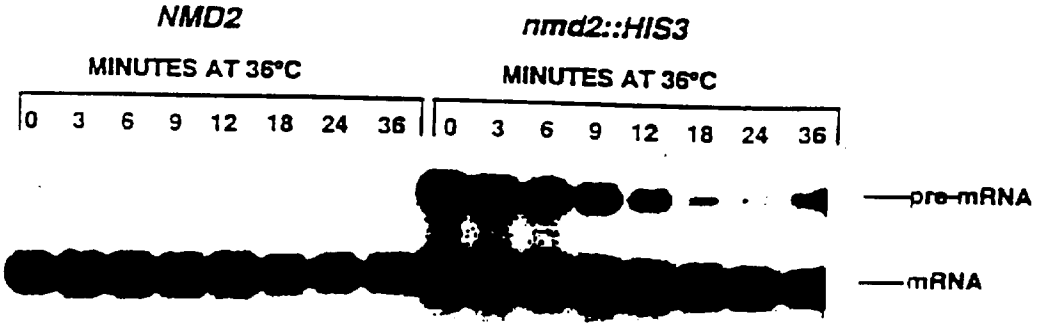


FIGURE 4C

FIGURE 5A

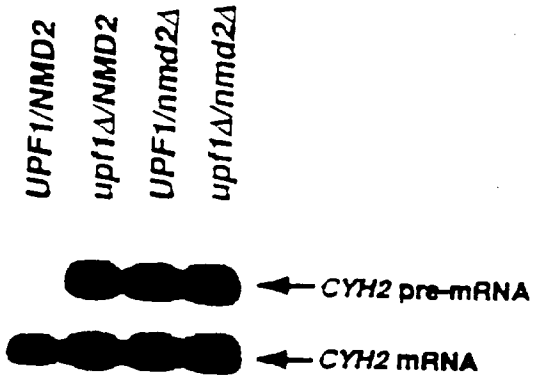


FIGURE 5B